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STUDIES ON AMINO ACID DIGESTION AND ABSORPTION IN GROWING PIGS

by

© Deirdre Margaret Conway

A THESIS

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.....

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Date....June 27, 1985.....

Dedication

To my parents, Patrick and Mary Conway.

Abstract

Experiments were conducted at the University of Alberta Large Animal Metabolic Unit using the technique of venous catheterization for determining blood amino acid concentrations. A total of twenty-four Yorkshire x Lacombe barrows (22 kg average liveweight) were surgically prepared with permanent indwelling portal vein catheters. The procedure involved the placement of a catheter in the portal vein thus allowing quantitative measurements of amino acid concentrations in the portal blood for an extended period of time.

In the first experiment, eight of the surgically prepared barrows were used to test duration of catheter patency and success of venous surgery. Patency success of venous catheterization was a function of the surgical procedure and the material that was used for catheter construction.

In the second experiment, the remaining sixteen barrows were used to determine the portal vein lysine concentrations when dietary lysine was supplied in the protein bound form or when a portion of the protein bound lysine was replaced with free lysine. The results indicate that there were no differences ($P>0.05$) in portal lysine concentrations or time of maximum lysine concentrations for plasma or whole blood when either form of lysine was fed.

The threonine requirement, based on average daily gain and feed conversion, for growing pigs (approximately 18 to 55 kg) was determined in the third experiment. The response in performance was measured in relation to stepwise additions of 0.05% threonine to a basal diet. Twenty-four pigs (12 females and 12 barrows) were used in each of five dietary treatments. The total threonine levels were as follows: 0.60, 0.65, 0.70, 0.75 and 0.79%. The digestibility of threonine was determined by the ileal and fecal analysis method prior to a growth trial with a total of 19 pigs. The ileal and fecal digestibilities of threonine were 56.9 and 79.1%, respectively. The total threonine requirement for growing pigs in the present studies ranged from 0.70 to 0.79%. Based on the supply of threonine, as determined by the ileal and fecal analysis method, the requirement ranged from 0.44 to 0.53% and from 0.57 to 0.66%, respectively. The present studies show the importance of taking into account the ileal

digestibility of threonine in the basal diet for an accurate determination of its requirement.

Key words: amino acid concentration, portal vein catheterization, free lysine, fecal and ileal amino acid digestibilities, threonine requirement, feed conversion efficiency, average daily gain, swine.

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I. Introduction

The partial replacement of a protein supplement(s) by a synthetic amino acid does not necessarily result in protein utilization similar to that obtained with the protein supplement(s). The utilization of free lysine by growing pigs fed once daily was lower than when the same amount and source of lysine was fed in six equal portions at three hourly intervals. It is suggested that this response is due to the differential rates of absorption of free lysine and protein bound lysine, resulting in an unbalanced supply of amino acids at the site of protein synthesis with infrequent feeding. Amino acid requirements are usually determined by measuring growth responses to the stepwise additions of the amino acid in question to a basal diet deficient in this amino acid. Consequently, if differences in the rate of absorption of free and protein bound amino acids exist, amino acid requirements may be over-estimated if once daily feeding systems are used. In addition to the previous consideration, amino acid requirements will be dependant on the digestibility of the amino acid in the basal diet that is used in the studies. In the past, the digestibilities of the amino acids were determined with the fecal analysis method. This method of determining amino acid digestibilities only considers the difference between the intake and the excretion in feces. However, this method does not take into account the modifying action of the the microflora in the large intestine on protein digestion and absorption. For these reasons, amino acid digestibilities should be determined with the ileal analysis method. Amino acid digestibilities determined by the ileal analysis method are based on the intake and the amount of amino acids passing through the distal part of the ileum in pigs fitted a simple-T or re-entrant cannula.

The objectives of the following studies were:

(1.) to prepare pigs with permanent indwelling portal vein catheters to determine whether lysine concentrations and time of maximum lysine appearance differed when either all the dietary lysine was supplied in the protein bound form or when a portion of the dietary lysine was supplied as free lysine, and

(2.) to determine the extent to which amino acid requirements (threonine) are dependant on

the digestibility of the amino acid in the basal diet.

II. Insertion of a Permanent Indwelling Portal Vein Catheter.

A. Introduction

Many methods have been employed for studying nutrient absorption in the pig. These include serial slaughter, intestinal cannulation and blood vessel catherization (Rerat, 1981). No method alone gives an unequivocal measurement of the amount of nutrient absorbed, only estimates can be obtained.

Recently, the measurement of protein digestion products (small peptides and individual amino acids) have been studied in the portal blood (Rerat et al., 1976). Permanent catheterization of the hepatic portal vein has allowed for measurements of changes in the post-prandial blood concentrations of nutrients, as virtually all absorbed nutrients pass through this vessel (Arsac and Rerat, 1962).

Changes in hepatic portal blood concentrations of amino acids reflect the simultaneous processes of digestion and absorption of the feedstuff and of metabolism occurring in the gastrointestinal tract wall. Therefore, the amino acid concentrations in the hepatic portal blood represent amino acids from both dietary and endogenous origin.

Research employing portal vein catheters has met with varied success. Problems associated with portal vein catheterization often result from catheter malfunction. However, catheter patency durations ranging from two weeks to several months (Herd and Barger, 1964; Shimada and Zimmerman, 1973; Withey et al., 1973; Knipfel et al., 1975) have allowed for extensive measurements of nutrient absorption in the hepatic portal blood.

The objective of the present experiment was to surgically prepare pigs with permanent indwelling portal vein catheters that would remain patent for at least 21 days and allow for continuous and painless blood sampling from conscious and unrestrained pigs.

B. Methods and Materials

Catheter Design

The catheter was prepared from 100 cm long polyvinyl chloride tubing (Argyle. St. Louis, MO, USA) with an outside diameter of 0.09 cm. Two cuffs, 5 mm in length with an outside diameter of 0.09 cm, were placed 3 and 5 cm distal to the catheter insertion tip (Figure II.1A). After the cuffs were put on the catheter, their positions were fixed by placing a drop of cyclohexanone on the slit in each cuff. This type of catheter preparation was previously described by Katz et al. (1969). The catheter tip was rounded to prevent damage to the vessel.

Animals

Eight Yorkshire x Lacombe barrows (20 kg average liveweight) were used in the experiment. The pigs were individually housed in adjustable stainless steel metabolic cages in an environmentally controlled barn with continuous light and an air temperature of 23 ± 1°C. An 18% crude protein pelleted starter diet (Table II.1) and water were supplied *ad libitum*. The pigs were allowed to adapt to the metabolic cages and new conditions for seven days prior to surgery.

Surgical Procedure

Feed was withheld from the pigs 24 hours prior to surgery. General anesthesia was induced with halothane (2%)-oxygen administered by inhalation through a face mask. Standard aseptic techniques and surgical procedures were employed.

The pig was placed supine on the operating table with its front and back legs tied to the table. The ventral region of the abdomen and the lateral aspect of the rib cage were shaved. All surgical fields were scrubbed with an iodine solution and rinsed with 70% ethanol. The entire pig was draped leaving only the area of the surgical incision exposed.

A midline incision, approximately 15 cm long, was made through the skin commencing approximately 2 cm below the xiphoid cartilage and extending approximately 2 cm distal to the umbilicus. The wound opening was enlarged by blunt dissection of adhering subcutaneous adipose tissue. The rectus abdominous muscle was opened by blunt dissection until the peritoneum was reached. This was followed by a 10 to 15 cm incision in the peritoneum. Thereafter, sterile saline (0.9% sodium chloride) was poured into the peritoneal cavity and around the wound edges. Saline moistened pads (Telfa®, 10 cm^2, Kendall Canada. Toronto, Ontario) were placed over the wound edges facilitating abdominal retraction and complete exposure of the abdominal contents.

The stomach and small intestine were repositioned to the left side of the peritoneal cavity and restrained. The lobes of the liver were raised and the common bile duct was seen in the hepata-duodenal ligament. The portal vein, close to the bile duct, continued cranially to enter the liver. The portal vein was located 3 to 5 cm caudal to the liver deep within the abdominal cavity. Once located, the vein was immobilized by placing a thumb and index finger around it. This also proved to be an effective method for arresting blood flow and preventing excessive blood loss when inserting the catheter. Upon isolation of the portal vein, a purse string suture of 3-0 silk was placed distal to the point of the portal vein entry into the liver and a #11 blade was used for making a small incision (2-3 mm long) in the vein through which the cuffed catheter was inserted.

Prior to insertion, the catheter tip was dipped in a 7% TDMAC heparin complex (Polyscience Inc. Warrington, PA, USA.) and filled with heparinized saline (40 units of sodium heparin/ml). The catheter was advanced through the lumen of the vein in the direction of blood flow for 3 cm or until the first cuff had been fully inserted. The purse string suture was tied to secure the catheter. An anchoring suture of 3-0 silk, placed 3 cm from the point of catheter entry into the vessel, was used to secure the catheter to the mesentery.

The catheter was placed loosely in the abdominal cavity and the liver and abdominal contents were returned to their midline positions. The catheter was exteriorized by drawing it

through a 1 cm incision in the muscle layer and tunnelling it subcutaneously through a stab wound between the fifth and sixth rib along the right lateral aspect. The insertion of the catheter in the portal vein is shown diagramatically in Figure II.2. A final check for haemostasis was made prior to closing the incision and 100 ml of sterile saline and 15 ml of oxytetracycline hydrochloride were placed in the peritoneal cavity.

The peritoneum and muscle layers were closed seperately using interrupted sutures of 2-0 chromic catgut. The skin was closed with an interrupted vertical mattress suture using 2-0 coated nylon, ensuring no overlap of the dermal layers in apposition.

The exteriorized catheter was flushed with heparinized saline, stop-cocked and placed in a polyethylene bag containing zepharin chloride. The polyethylene bag was further placed in a cotton bag which was sutured to the side of the pig.

Upon completion of surgery, the pig was immediately returned to the metabolic crate and allowed to recover. The pigs received 4 ml of oxytetracycline hydrochloride intramuscularly for 5 days following surgery. The starter diet and water were supplied *ad libitum* to the pigs for 6 days. Thereafter, the pigs were fed 1000 g of the experimental diet once daily at 0800 hours for 2 weeks.

Blood Collection

Blood was collected from each pig after feeding on days 7 and 14 of the experimental period at various intervals for a 24 hour duration. The catheter was removed from the polyethylene and cotton bags and the adaptor was rinsed with sterile saline. A 10 ml syringe was attached to a 16 gauge hypodermic needle and 2 ml of heparinized saline was slowly infused into the catheter to prompt blood flow. A 1 ml volume of blood was withdrawn and discarded. A second sterile needle and syringe were used to collect 10 ml of blood. After the blood was withdrawn, the catheter was flushed with 2 ml of heparinized saline to remove remaining traces of blood and to prevent the catheter from plugging. A final 3 ml of heparinized saline was infused into the catheter until further blood sampling. Packed cell volume determinations were

made following each blood sampling.

C. Results and Discussion

The entire surgical procedure was completed within one hour. The pigs regained consciousness within 15 minutes after discontinuing the anesthesia by which time they had been returned to their metabolic crates. Recovery from surgery was rapid. The pigs consumed the starter diet and drank water within 10 hours after surgery. Three days after the completion of surgery the pigs were consuming 1000 g of the starter diet.

Post-surgical complications were encountered. These included intestinal hernias and infections. Intestinal hernias were the result of either an inadequate number of sutures used in closing the abdominal muscle layers or suture breakage along the muscle layer. In both cases, the viscera weight caused the intestines to protrude through the peritoneum and between the muscle and the dermal layers and consequently, repeated surgery was required. The dermal and muscle layers were reopened along the original incision line and the intestines repositioned. Closure of the dermal layer was as previously described. Adhesion formation between the muscle and dermal layers was rapid and therefore repeated surgery was performed quickly in order to reduce any further tissue damage.

As with all permanent indwelling venous catheters, infection was of prime concern. After approximately ten days post-surgery, localized skin infections appeared at the point of exteriorization of the catheter; these were characterized by the formation of pus. This was difficult to avoid because this is the point of catheter contact to the external environment. To minimize infections the skin area surrounding the catheter exit from the body wall was cleansed with warm water and a dilute 3.0% hydrogen peroxide (H_2O_2) solution twice daily.

In general, the post-surgical complications were not characterized by vomitting or inappetence. However, one animal showed signs of inappetence and weight loss and died. A post-mortem examination revealed intestinal strangulation by the catheter. As a result, the small intestine had atrophied and the stomach was distended. Thereafter, extra precaution was

taken to prevent the catheter from twisting and causing intestinal blockage by ensuring proper alignment of the viscera and catheter prior to abdominal closure.

Subcutaneous wound healing was rapid. The length, depth and breadth of the incision were all favourable to enhance trouble free healing of the cutaneous wound. Ordman and Gillman (1966) suggest that in pigs the dermal part of an incision is sealed off from the external environment by new epithelial cells within 24 hours of surgery. This was clearly evident 24 hours following the insertion of the portal vein catheter.

The portal vein was adequately exposed through manipulation of the small intestine and the liver. However, because of the depth of the portal vein within the abdominal cavity and the small surgical workspace, it was often necessary to exteriorize a considerable portion of the small intestine. This presented a complication because once exposed, the small intestine dried rapidly and adhesion between sections of the small intestine resulted. However, this problem was avoided by placing saline soaked gauze over the exposed intestine and pouring saline into the abdominal cavity prior to closure.

Difficulty arose during surgery when there was excessive loss of blood through the portal vein incision, making catheter insertion difficult, if not impossible. The polyvinyl chloride catheter did not remain rigid and as a result, the catheter became soft and pliable thus making insertion difficult. However, once inserted, the purse string suture around the catheter was tightened and the catheter was secured in the portal vein. An anchoring suture placed 3 to 5 cm at the end of catheter entry into the vein was necessary to ensure the security of the catheter in the abdominal cavity.

Duration of catheter patency varied. The maximum patency attained using this type of polyvinyl chloride catheter was 28 days, with an average patency of 15 days. The most common cause of patency loss was clot formation at the tip of the catheter. However, on occasion catheter patency could be re-established by threading a small sterilized wire guide, the same length as the catheter, through the catheter to dislodge any clots that had formed on the tip.

The material used to construct the catheter may have caused the short patency duration. Prolonged patency has been suggested when catheters are constructed from Silastic® (Dow Corning Canada Inc. Mississauga, Ontario) tubing rather than from polyvinyl chloride or polyethylene chloride tubing (Withey et al., 1973). The prolonged patency duration is believed to be due to the chemical nature of the Silastic® material. Silastic® has the advantage of being virtually non-reactive to body fluids and tissues because it is an extremely inert material (Doering et al., 1967). As a result, Silastic® tubing is very resistant to clotting, sticking and incrustation and consequently, patency and blood flow are relatively easily maintained (Sherwood, 1965). In the future, the catheter will be constructed from polyvinyl chloride with the addition of an 8 to 10 cm Silastic® covering over the insertion end of the catheter (Figure II.1B). This will, to some extent, eleviate the problems of incrustation and clot formation over the catheter tip and prolong patency for up to two months (R.J. Early; personal communication).

The catheter design incorporates an important feature. The fixed cuffs, along with the purse string suture, were used to immobolize and secure the catheter in the vein. However, this design feature may be difficult to use if the catheter was inserted into the portal vein through a branch of the anterior mesenteric vein. Suturing a cotton pouch to the side of the pig was an effective means of both keeping the catheter clean and preventing the pig from physically disrupting the implanted catheter. This preparation caused no apparent pain to the animal and infection did not result.

Post-mortem examinations were performed on pigs in which catheter patency had been lost. With the one exception that was previously mentioned, there were no signs of intestinal adhesions or blockage. The catheter remained secure in the portal vein and the area showed little evidence of surgical trauma. Post-mortem examinations of pigs, in which patency had been lost, showed that a fibrous clot had formed around the catheter tip or inside the catheter. This problem may be avoided in future surgical preparations by using a Silastic® tipped polyvinyl chloride catheter. In general, post-mortem examinations revealed that the surgical

procedures caused little internal trauma to the pig. Other investigators have also reported that surgery of this nature causes little physical trauma to the animal (Rerat et al., 1980).

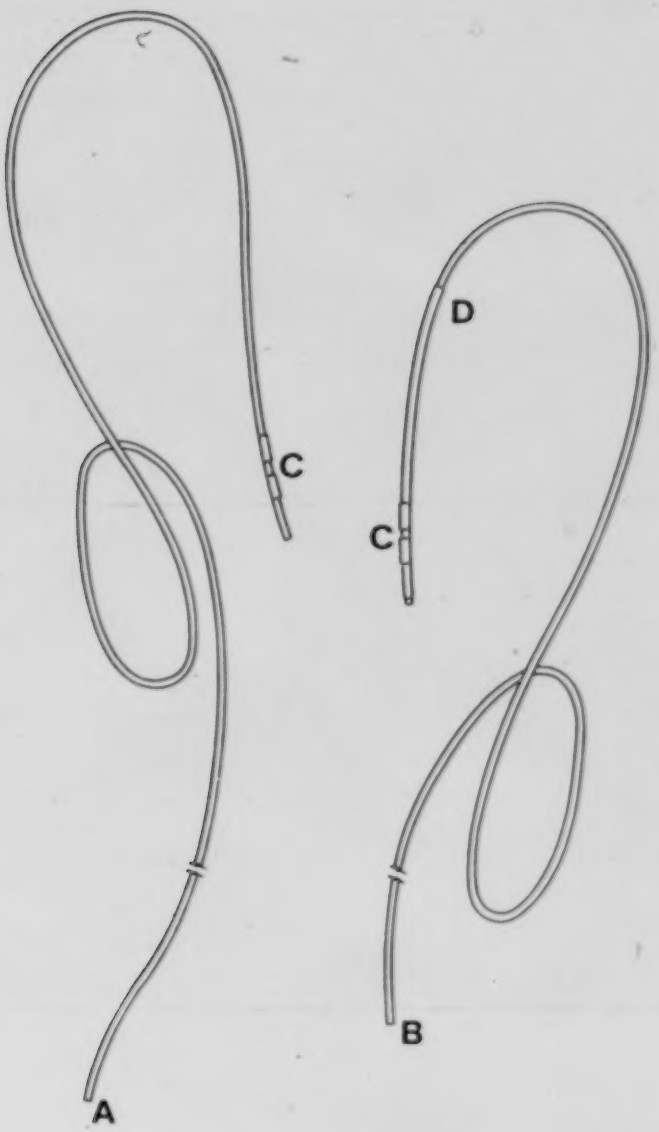
At the end of the experiment, the remaining pigs were returned to communal pig pens. The cotton catheter pouch was removed by cutting the sutures. The catheter was removed by pulling it taut and burning the catheter (thus closing it) close to the pigs' body.

Continuous or discontinuous blood sampling using permanent polyvinyl chloride catheters appears to be a valuable tool for the chronological estimation of the appearance, concentration and tissue distribution of nutrients or other substances (for example, drugs) and can be applied to pigs of any size. However, there is a need to find new material for catheter construction which will allow for blood collection of longer than a two week duration.

Table II.1 Formulation of the starter diet.

Ingredients, %	
Wheat	25.0
Barley	25.0
Oat groats	25.0
Soybean meal	18.0
Tallow	3.0
Dicalcium phosphate	1.5
Calcium carbonate	1.0
Mineral/vitamin mix[1]	1.0
Iodized salt	0.5

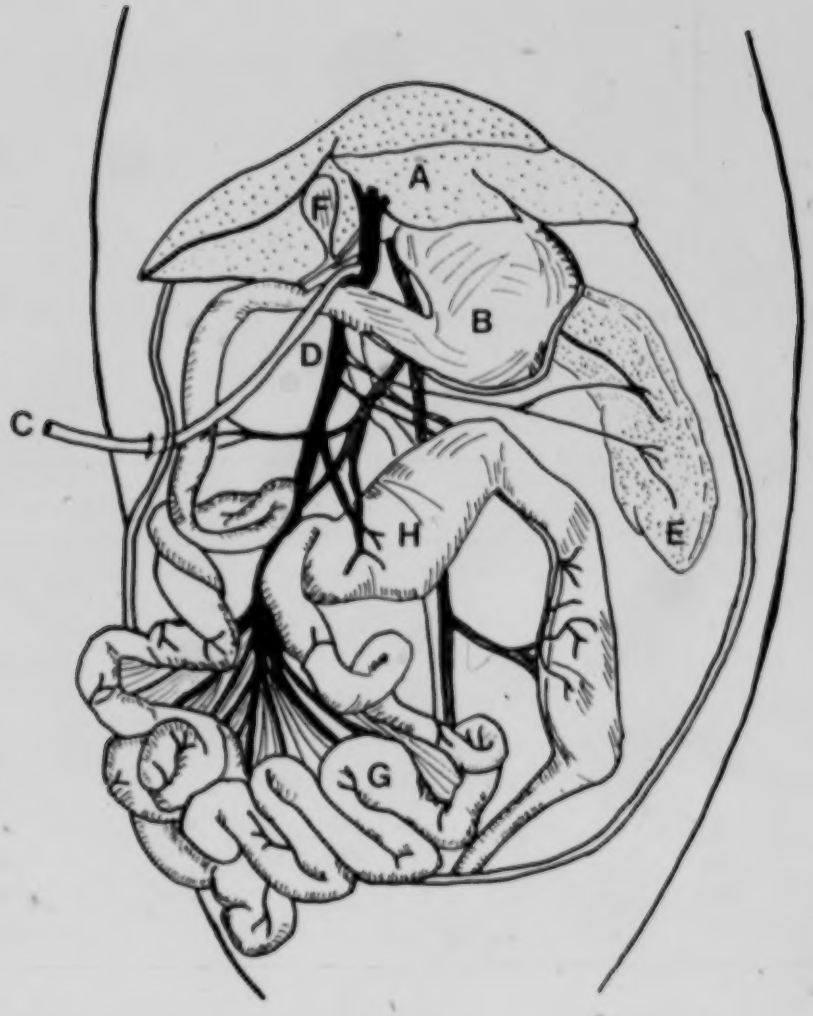
[1]The mineral/vitamin mix provided the following per kilogram of diet: 120.0 mg zinc; 48.0 mg manganese; 100.0 mg iron; 10.0 mg copper; 0.1 mg selenium; 7500 IU vitamin A; 700 IU vitamin D; 45 IU vitamin E; 12 mg riboflavin; 40 mg niacin; 27 mg calcium pantothenate; 28 μg vitamin B_{12}.



A - polyvinyl chloride catheter; B - Silastic® tipped polyvinyl chloride catheter;

C - fixed cuffs; D - Silastic® tubing

Figure II.1 Portal vein catheters.



A-liver; B-stomach; C-catheter; D-portal vein; E-spleen; F-gall bladder

G-small intestine; H-large intestine.

Figure II.2 Placement of a cuffed catheter in the portal vein of a pig.

D. Literature Cited

Arsac, M. and A.A. Rerat. 1962. Technique de fistulation de la veine porte chez le porc. Annls Biol. Anim. Biochim. Biophys. 2:335-343.

Doering, R.B., Stemmer, E.A. and J.E. Connolly. 1967. Complications of indwelling venous catheters with particular reference to catheter embolus. Amer. J. Surg. 114:259-266.

Early, R.J. 1984. Unpublished results. Department of Animal Science. University of Alberta. Edmonton, Alberta, Canada.

Herd, J.A. and A.C. Barger. 1964. Simplified technique for chronic catheterization of blood vessels. J. Applied Physiology 19:791-792.

Katz, M.L. and E.N. Bergman. 1969. A method for simultaneous cannulation of the major splanchnic blood vessels of sheep. Am. J. Vet. Res. 30:655-661.

Knipfel, J.E., Peace, R.W. and J.A. Evans. 1975. Multiple vascular and gastric cannulation of swine for studies of gastrointestinal, liver and peripheral tissue metabolism. Lab. Anim. Sci. 25:74-78.

Ordman, L.J. and T. Gillman. 1966. Studies in the healing of cutaneous wounds. 1. The healing of incisions through the skin of pigs. Arch. Surg. 93:857-882.

Rerat, A.A., Corring, T. and J.P. Laplace. 1976. Protein Digestion and Absorption. In: D.J.A. Cole, K.N. Boorman, P.J. Buttery, D. Lewis, R.J. Neale and H. Swan (Eds.) Protein Metabolism and Nutrition. Butterworths, London, England.

Rerat, A.A., Vaugelade, P. and P. Villiers. 1980. A new method for measuring the absorption of nutrients in the pig: a critical examination. In: A.G. Low and I.G. Partridge (Eds.) Current Concepts of Digestion and Absorption in Pigs. National Institute for Research in Dairying, Reading, England.

Rerat, A.A. 1981. Digestion and absorption of nutrients in the pig. Wld. Rev. Nutr. Diet. 37:229-287.

Sherwood, N.S. 1965. Experience with a new silastic catheter. J. Urol. 93:307-308.

Shimada, A. and D.R. Zimmerman. 1973. Hepatic portal catheterization and comparison of portal blood and anterior vena cava free plasma amino acid patterns in the baby pig. J. Anim. Sci. 36:245-251.

Withey, R.J., Willes, R.F., Evans, J. and F.R. Bryce. 1973. The surgical preparation and maintenance of pigs in bioavailability studies. Lab. Anim. Sci. 23:122-125.

III. Effects of Dietary Lysine on Blood Lysine Concentrations in Growing Pigs.

A. Introduction

The partial replacement of protein supplements by a synthetic amino acid in cereal based diets (until the next amino acid becomes limiting) does not necessarily result in protein utilization or pig performance similar to that obtained with the protein supplements. Rerat et al. (1976) suggest that this may be due to differences between the rate of absorption of the free amino acids compared with amino acids consumed in the form of protein.

Buraczewska et al. (1978) and Buraczewska et al. (1980) showed that when pigs were fed a basal diet supplemented with synthetic lysine, the rate of passage of the synthetic lysine from the stomach to the small intestine was faster than that of the dietary protein. These results agree with those previously cited by Rolls et al. (1972) who showed that in studies with rats, free supplemental amino acids were absorbed faster than those derived from the protein source.

Batterham (1974) and Batterham and O'Neill (1978) have shown that the utilization of free lysine by growing pigs fed once daily was lower than when the same amount and source of lysine was fed in six equal portions at three hourly intervals. It was suggested that infrequent feeding resulted in differential rates of absorption of free lysine and protein bound amino acids, resulting in an unbalanced supply of amino acids at the site of protein synthesis.

It is generally assumed that the inclusion of free amino acids into a grower pig diet results in the full utilization of the amino acids by the pig and therefore requirements are routinely determined by growth responses to the addition of the supplemental amino acids. However, if differences in the absorption of free and protein bound amino acids do exist, amino acid requirements based on this criterion may be over-estimated if once daily feeding systems are used. In addition, the high expense of incorporating synthetic amino acids into diets would make it nutritionally and economically desirable to determine a means by which a larger portion of the supplemental amino acid could be utilized.

The objective of the present study was to determine whether lysine concentrations and time of maximum lysine appearance in blood from the portal vein differed when either all the dietary lysine was supplied in the protein bound form or when a portion of the dietary lysine was supplied as free lysine.

B. Materials and Methods

Animals

Sixteen Yorkshire x Landrace barrows (22 kg average liveweight) were surgically prepared for blood collection. The procedure involved the insertion of a permanent indwelling polyvinyl chloride catheter into the portal vein. Following surgery the pigs were individually housed in stainless steel metabolic crates in an environmentally controlled barn (continuous light and an air temperature of 23 ± 1°C) and allowed a 7 day recuperation period. During this time, the pigs were trained to consume 1000 g of an experimental diet within a one hour period. Water was supplied *ad libitum* from a low pressure drinking nipple.

The sixteen animals were used in a complete randomized block design and fed 1000 g of an experimental diet once daily at 0800 hours for a 14 day period. The formulation of the diets and proximate and amino acid analyses are shown in Tables III.1 and III.2, respectively. The diet allowances were adjusted every three days, increasing by 100 g per 2.5 kg liveweight gain, after individually weighing each pig. Both diets were formulated to meet the National Academy of Sciences-National Research Council (NAS-NRC 1979) recommended nutrient requirement levels and were fed on an equal lysine basis. Diet 1 supplied all the NAS-NRC (1979) lysine requirements in the protein bound form whereas diet 2 supplied a portion of the lysine in the synthetic form, at the expense of soybean meal.

Blood Collection

Blood was collected from each pig on days 7 and 14 (catheter patency permitting) of the experimental period. Blood was sampled over the whole periprandial period. Collections were performed at one hour and one half hour prior to feeding at 0800 hours. Immediately after feeding blood collections were continued at half hour intervals for a period of 4 hours. Between 1200 hours and 1500 hours blood collections were performed at one hour intervals; from 1500 hours until 2100 hours, blood collections were taken at 3 hour intervals. Three final blood collections were performed at 2400, 0400 and 0800 hours.

A 10 ml volume of blood was drawn into a heparinized syringe for each collection and a haematocrit value (PCV) was immediately determined. Whole blood (3 ml) was transferred into a 5 ml plastic vial containing approximately 20 mg of sodium fluoride and stored at -23°C until further analysis. The remaining whole blood (7 ml) was centrifuged at 2,500 x g at 4°C for 10 minutes and approximately 3 ml of plasma were decanted into a vial containing approximately 20 mg of sodium fluoride and stored at -23°C until further analysis.

Analytical Procedures

Analysis of protein (N x 6.25) in the diet was determined by the Kjeldahl method of analysis according to the Association of Analytical Chemists (AOAC, 1980). Blood samples were thawed at 21°C three hours prior to analyses. Blood and feed amino acids were determined by gas liquid chromatography according to the methods described by Pearce (1981).

Reagents

An amino acid standard solution for protein hydrolysates (2.5 umole/ml of each amino acid; AA-5-18), L-norleucine (internal standard) and Dowex 50W 1-X8 cation exchange resin (200-400 dry mesh, H^+ form) were purchased from Sigma Chemical Company (St. Louis, MO, USA). Heptafluorobutyric anhydride (HFBA) was obtained from Pierce Chemical Company (Rockford, IL, USA). Isobutanol-3N HCl was redistilled before use and stored at room

temperature in a pyrex reagent bottle with a ground glass stopper. Other reagents were prepared according to standard laboratory techniques.

Standards

A 10 mM stock solution of L-norleucine was prepared and stored at 4°C until required, at which time a 100 uM working solution served as an internal standard. A 100 uM amino acid standard solution was prepared, stored at 4°C and used as needed.

Cation Exchange Resin Preparation

The cation exchange resin preparation was modified from the procedures described by Kaiser et al. (1974). Equal volumes of cation exchange resin and deionized water were mixed and stirred for 30 minutes. The water was decanted and an equal volume of 2N NaOH was added. The resin slurry was stirred for 30 minutes, washed thrice with deionized water and 2N NaOH was again added. The cation exchange resin was washed ten times with deionized water to remove the alkali and to attain a neutral pH as measured on a Beckman Model 4500 digital pH meter. The regeneration of the resin to its H^+ form was accomplished by adding an equal volume of 2N HCl and stirring for 1 hour. The addition of 2N HCl to the resin was repeated twice. Volumes (200 ml) of deionized water were used to wash the cation exchange resin until neutrality was reached. The cation exchange resin preparation was stored at room temperature in a capped glass bottle.

Cation Exchange Column

A 23 mm Pasteur pipette served as a cation exchange column into which a glass wool plug and approximatelty 200 ul of cation exchange resin were added. The column was washed once with deionized water prior to the addition of a sample. After the samples had passed through the column, the column was discarded.

Cation Exchange Clean Up

Equal volumes (0.5 ml) of 100 uM norleucine and plasma or whole blood were added to 16 x 100 mm Borosilicate glass disposable culture tubes. The sample was deproteinized by the addition of 4 ml of 10% TCA. The sample was centrifuged (2,500 x g at 4°C for 10 minutes) to precipitate blood protein. The supernatant was passed through the cation exchange column which was then washed once with distilled water. The effluent was discarded. Two additions of 2 ml of NH_4OH were passed through the column and the effluent containing free amino acids was collected in a 13 x 100 mm screw cap culture tube.

Derivative Preparation

Preparation of the derivatives was modified from the procedures described by Pearse (1977). All derivatization steps were performed in 13 x 100 mm screw cap culture tubes with TFE lined caps. Excess reagents were evaporated in an oven at 110°C. When required, nitrogen was used for purging the headspace of the vial.

The 4 ml NH_4OH effluent collected in the culture tubes was dried by evaporation for 12 hours in a 110°C oven. Isobutanol-3N HCl (0.5 ml) was added and after purging the headspace, the vial was capped and heated in a 110°C oven for 1 hour. After cooling to room temperature, the cap was removed and excess reagent was evaporated. Heptafluorobutyric anhydride (100 ul) was added, the headspace was purged and the vial was capped and heated at 110°C for 30 minutes. The sample was then cooled at 4°C for 15 minutes prior to injecting 1 to 5 ul onto the capillary column.

Chromatography

Amino acid analyses were performed using a Varian model 3700 gas chromatograph fitted with a flame ionization detector and containing a 30 m x 0.25 mm fused silica SE-30 capillary column (film thickness, 0.25 um; J and W Scientific Inc., Rancho Cordova, CA, USA). Injector and detector temperature were both 250°C. The oven temperature was

programmed to rise from 100° to 265°C at a rate of 6°C per minute. A 1:50 split injection mode was used; the carrier gas (nitrogen) flow rate was 0.75 ml per minute through the column and the make up gas (nitrogen) flow rate was 30 ml per minute through the detector. The air flow rate and the hydrogen flow rate to the FID were 300 and 30 ml per minute, respectively.

Retention times and peak areas were obtained using a Hewlett-Packard Series 3353 Laboratory Automation System (Avondale, PA, USA). Relative molar response factors (RMR) and relative concentrations were calculated as the ratio of amino acid peak area to the norleucine internal standard peak area. The areas were graphically recorded using a Fisher Recordall Series 5000 recorder (Fisher Scientific Co., Edmonton, ALTA, CANADA).

Feed Protein Hydrolysis

Fifty mg of feed, ground through a 1 mm screen, were weighed into a 13 x 100 mm screw cap culture tube. Five ml of redistilled 6N HCl were added, the culture tube was purged, and capped tightly with a TFE lined cap.

The feed samples were hydrolyzed in a forced-draft oven at 110°C for 24, 48 and 72 hours. Upon completion of hydrolysis, 0.5 ml of 10 mM norleucine internal standard was added to the cooled hydrolysate. The sample was transferred to a 125 ml round bottom flask and dried under reduced pressure at 70°C with a rotary evaporator. The dry residue was reconstituted with 10 ml of distilled water and stored at -23°C until amino acid analysis was performed.

Amino Acid Analysis

A 200 *u*l aliquot of the feed hydrolysate or of the amino acid standard was placed into a 13 x 100 mm screw cap culture tube. The sample was dried at 110°C in a forced-draft oven. Isobutanol-HCl (0.5 ml) was added to the dried residue, the tube was purged with nitrogen gas, tightly capped and heated in a forced-draft oven at 110°C for 30 minutes. Following esterification of the amino acids, the isobutanol-HCl solvent was removed by evaporation at

110°C. Heptafluorobutyric anhydride (100 ul) was added, the tube purged with nitrogen gas, capped tightly and heated at 110°C for 30 minutes. The solution was cooled to 4°C before 1 ul was injected onto the gas chromatograph.

Statistical Analysis

The data were statistically analyzed using a multiple regression calculation on the time of maximum lysine concentration. A quadratic equation was used to obtain a best fit for the data. A two-way analysis of variance was used to test for significant differences between time and lysine concentration in plasma and whole blood.

C. Results and Discussion

The results indicate that the time at which the maximum concentration of lysine in the portal vein was reached was not significantly ($P>0.05$) different when lysine was fed in the protein bound or free form. The portal concentrations of lysine with respect to time were scattered throughout the observation period and showed no definite response patterns to the source of lysine. The peak plasma lysine concentrations of pigs fed protein bound ($n=4$) and free lysine ($n=6$) were 349 $\pm$50 and 633 $\pm$125 umole per litre and were reached at 117 and 104 minutes after feeding, respectively. The peak whole blood lysine concentrations of the protein bound and synthetic lysine were 395 $\pm$56 and 436 $\pm$63 umole per liter and were reached at 109 and 116 minutes after feeding, respectively (Figure III.1).

In studies with rats, Goldberg and Guggenheim (1962) observed that the maximum amino acid concentration in the plasma of the portal vein was obtained 30 minutes after the ingestion of a protein meal. Rerat et al. (1976) suggested that in pigs, the increase in the level of circulating amino acids can be detected as early as 30 minutes after the ingestion of a protein meal depending on the quantity and nature of the protein fed. In the present experiment, maximum plasma and whole blood concentrations of lysine peaked at approximately 110 minutes after feeding and declined linearly, reaching their lowest concentrations or baseline

values between 7 to 12 hours after feeding.

There are conflicting reports on the relative rate of absorption of protein bound and free amino acids. In rats, Gupta et al. (1958) and Rogers et al. (1960) found that protein bound and free amino acids were absorbed at approximately the same time. In contrast, Rolls et al. (1972) showed that in rats the absorption of free amino acids was faster than amino acids in protein. Similarly, Waltz (1972) showed that in pigs fed an oat based diet, synthetic lysine was absorbed faster than amino acids in protein.

In the present study, there was considerable variation in the results among pigs and between pigs on the same dietary treatment, making the results rather difficult to interpret. Possible explanations for the variations may be due to the individual pig responses to the diets. Between treatment variation in lysine concentrations in the plasma from the portal vein were expected due to the different lysine sources in the diets.

The products of protein digestion are absorbed and transported in the blood to various tissues as free amino acids or small peptides. Therefore, levels of free amino acids in the portal plasma would be expected to be a reasonably accurate reflection of the dietary proteins and amino acids that were digested and presumably made available to the pig. However, amino acid utilization is dependant upon the physiological state of the animal and on the buffering capacity of the body's metabolic pool. For example, increases in tissue protein synthesis result in the removal of free amino acids from the circulating blood, whereas protein catabolism causes a temporary increase in the level of free amino acids (Pion, 1976). A further consideration is that plasma is a dynamic tissue and the amino acid concentration measured at any one time is a balance between the supply and the removal of amino acids from the blood (McNab, 1980).

Blood flow rates increase after feeding (Braude et al., 1970) and a concomitant increase in the removal of amino acids from the small intestine without measurable differences in the amino acid concentrations in the portal blood are posssible. Therefore, caution is needed when interpreting plasma amino acid concentrations in the portal blood.

Surgical preparations involving catheter insertion in the portal vein are unlikely to cause serious physical trauma to the pig (Rerat et al., 1980). However, when blood samples are withdrawn, the presence of a sampler may place a stress upon the pig resulting in the breakdown and liberation of the amino acid from sources other than those of dietary origin. Blood flow measurements were not made and radio-isotopes were not used, therefore conclusions on the source of lysine (that is, free lysine, protein bound lysine or the release of lysine from tissues or endogenous sources) cannot be made.

When conducting experiments using blood parameters, consideration must be given to the quantity of blood withdrawn from the animal per experimental period, as well as the recovery time required between experiments. The use of discontinuous sampling in the present study required that a large volume of blood (10 ml) be withdrawn per sample. As a result of the rapid increase in portal amino acid concentration during digestion, and in order to obtain an accurate evaluation of the changes in amino acid concentration with time, it was necessary to have closely spaced sampling intervals for the first four hours after feeding. The length of time needed for the digestion of some proteins (Pion et al., 1964) required continuous sampling over a 24 hour period. A sampling duration of this length, and the volume of blood required per sample, resulted in the removal of a total of at least 250 ml of blood from each pig during each test period. The haematocrit value progressively declined from approximately 40 to 30 (on average) over the 24 hour sampling duration. Therefore, in order to keep the animal in a good nutritional status, the haematocrit was monitored and when the haematocrit returned to its normal level, usually requiring five to seven days, the next set of samples were taken. These procedures agree with those of Rerat et al. (1980) who suggested that after the removal of 250 ml of blood, the haematocrit declines thus necessitating a rest period of at least two days before further experimentation is begun.

In the pig, the relationship between dietary lysine concentration relative to plasma lysine concentration varies (Windels et al., 1971; Davey et al., 1973). Davey et al. (1973) found that the patterns of plasma lysine responses were inconsistant with the dietary protein

intake of pigs at various ages. In the present study, the lysine concentrations reported in the plasma were also variable, however peak concentrations were found at specific times after feeding. The portal vein drains the small and large intestine, spleen and pancreas and it is considered to be the main pathway of amino acid absorption (Rerat et al., 1976). Therefore, changes in lysine concentrations in this vein should accurately reflect those amino acids of dietary origin.

Studies involving quantitative measurements of blood parameters (Puchal et al., 1962; Mitchell et al., 1968; Typpo et al., 1970; Stockland et al., 1970; Rerat et al., 1980) are difficult to make because diverse surgical preparations and analytical methods exist. Venous catheterization, as with other methods of measuring amino acid concentration, has limitations. It is complex and time consuming because extensive surgical preparation of the experimental animal is required before quantitative measurements can be made. It gives no indication of the amino acids absorbed and metabolized by the intestinal cell wall and hence not appearing in the portal blood. A further consideration is that a distinction cannot be made between amino acids of endogenous or dietary origin although tentative measurements have been suggested (Rerat, 1973).

In the present experiment there were no significant ($P>0.05$) differences between the concentration and appearance of lysine and dietary treatment in whole blood or in plasma. This suggests that free lysine, as a portion of the total dietary lysine, appears in the portal blood at approximately the same time as protein bound lysine. While variation in the portal blood lysine concentration between animals on the same treatment and within animals on the same treatment must be recognized, of more importance may be the appearance of lysine at approximately the same time for both of the dietary treatments.

Table III.1 Formulation of the experimental diets.

	Diet 1	Diet 2
Ingredients, %		
Barley	50.0	50.0
Wheat	31.5	31.5
Soybean meal	15.0	7.5
Cornstarch	—	7.22
Calcium carbonate	1.0	1.0
Dicalcium phosphate	1.0	1.0
Mineral/vitamin mix[1]	1.0	1.0
Iodized salt	0.5	0.5
Lysine HCl	—	0.28

[1]The mineral/vitamin mix provided the following per kilogram of diet: 120.0 mg zinc; 48.0 mg manganese; 100.0 mg iron; 10.0 mg copper; 0.1 mg selenium; 7500 IU vitamin A; 700 IU vitamin D; 45 IU vitamin E; 12 mg riboflavin; 40 mg niacin; 27 mg calcium pantothenate; 28 μg vitamin B_{12}.

Table III.2 Proximate and amino acid analyses of the experimental diets.

	Diet 1	Diet 2
Chemical analyses, %		
Dry matter	90.9	91.1
Crude protein, N x 6.25	18.2	18.0
Ether extract	1.2	1.3
Ash	5.1	5.1
Energy (kj/g)	16.3	16.3
Amino acids[1], %		
Indispensable[2]		
Arginine	0.95	0.98
Histidine	0.42	0.38
Isoleucine	0.63	0.62
Leucine	1.22	1.16
Lysine	0.82	0.83
Methionine	0.21	0.22
Cystine	0.17	0.18
Phenylalanine	0.83	0.81
Threonine	0.62	0.59
Tyrosine	0.42	0.42
Valine	0.68	0.70
Dispensable		
Alanine	0.66	0.68
Aspartic acid	1.39	1.32
Glutamic acid	3.56	3.51
Glycine	0.68	0.68
Proline	1.13	1.07
Serine	0.81	0.77

[1]Expressed on a dry matter basis.
[2]Tryptophan was not determined.

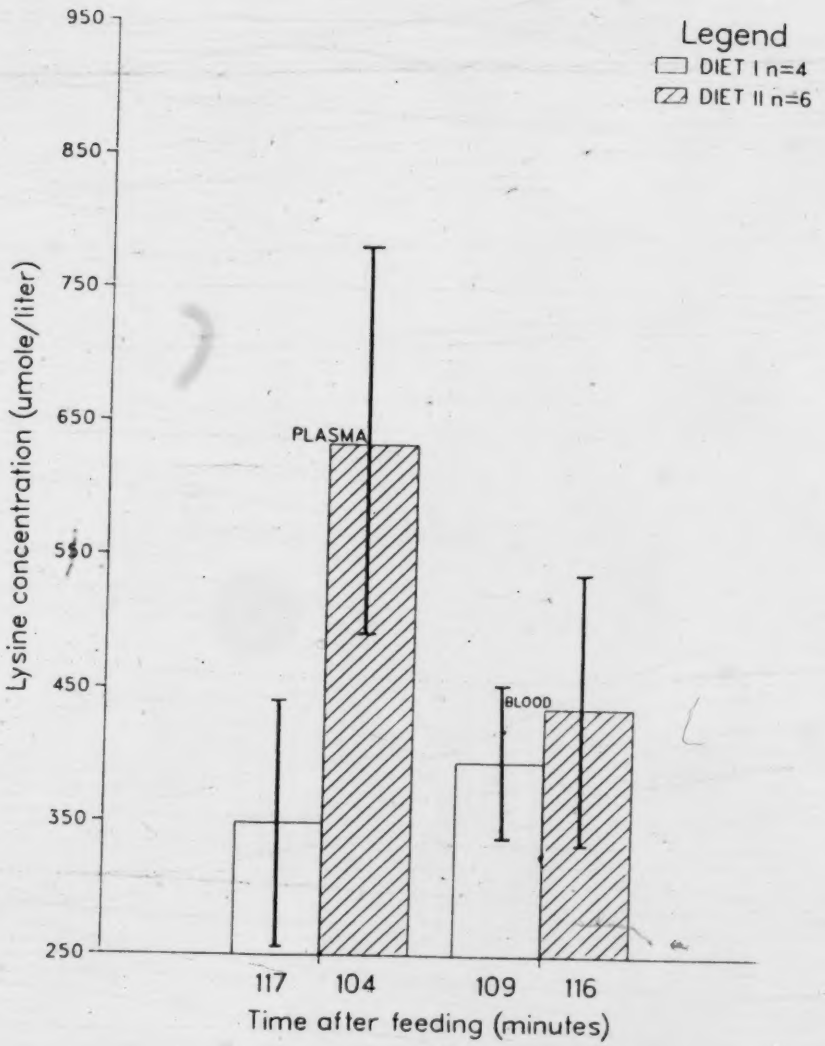


Figure III.1 Peak lysine concentrations in the portal blood following a meal.

D. Literature Cited

A.O.A.C. 1980. Offical Methods of Analysis. (13th Ed.) Association of Offical Analytical Chemists. Washington, D.C., U.S.A.

Batterham, E.S. 1974. The effect of frequency of feeding on the utilization of free lysine by growing pigs. Br. J. Nutr. 31:237-242.

Batterham, E.S. and G.H. O'Neill. 1978. The effect of frequency of feeding on the response by growing pigs to supplements of free lysine. Br. J. Nutr. 39:265-270.

Braude, R., Cutts, I.R., Myers, A.W. and J.W.G. Porter. 1970. Measurement of amińo acid levels in portal blood plasma of pigs and of rate of portal blood flow. Proc. Nutr. Soc. 29:60 Abstract.

Buraczewska, L., Zebrowska, T. and S. Buraczewski. 1978. The rate of passage of synthetic lysine and dietary protein from the stomach to the intestine in pigs. Rocz. Nauk. Roln. Ser. B Zootech. 99:107-113.

Buraczewska, L., Lachowicz, J. and S. Buraczewski. 1980. The rate of absorption of synthetic lysine and dietary protein in the upper half of the small intestine in the pig. Arch. Tierernaehr. 30:751-758.

Davey, R.J., Phelps, J.W. and C.H. Thomas. 1973. Plasma free amino acids of swine as influenced by diet protein level, animal age and time of sampling. J. Anim. Sci. 37:81-86.

Goldberg, A. and K. Guggenheim. 1962. The digestive release of amino acids and their concentrations in the portal plasma of rats after protein feeding. Biochem. J. 83:129-135.

Gupta, J.D., Dakroury, A.M. and A.E. Harper. 1958. Observation on protein digestion *in vivo*. 1. Rate of disapearance of ingested protein from the gastrointestinal tract. J. Nutr. 64:447-456.

Kaiser, F.E., Gehrke, C.W., Zumwalt, R.W. and K.C. Kuo. 1974. Amino acid analysis. Hydrolysis, ion-exchange cleanup, derivatization and quantitation by gas-liquid chromatography. J. Chromat. 94:113-133.

McNab, J.M. 1980. The concept of amino acid availability in farm animals. In: W. Haresign and D. Lewis (Eds.) Recent Advances in Animal Nutrition. Buttersworth, London, England.

Mitchell, Jr., J.R., Becker, D.E., Jenson, A.H., Harmon, B.G. and H.W. Norton. 1968. Determination of amino acid needs of the young pig by nitrogen balance and plasma-free amino acids. J. Anim. Sci. 27:1327-1331.

National Research Council. 1979. Nutrient requirements of swine. Eighth revised edition. Washington, D.C., U.S.A.

Pearce, R.J. 1977. Amino acid analysis by gas liquid-chromatography of N-heptafluorobutryl isobutryl isobutyl esters. Complete resolution using a support-coated open tubular capillary column. J. Chromat. 136:113-126.

Pion, R., Fauconneau, G. and A.A. Rerat. 1964. Variation in portal amino acid levels in the course of digestion of the pig. In: H.N. Munro (Ed.) The Role of the Gastrointestinal Tract in Protein Metabolism. Blackwell, Oxford, England.

Pion, R. 1976. Dietary effects and amino acids in tissues. In. D.J.A. Cole, K.N. Boorman, P.J. Buttery, D. Lewis, R. J. Neale and H. Swan (Eds.) Protein Metabolism and Nutrition. Buttersworth, London, England.

Puchal, F., Hays, V.W., Speer, V.C., Jones, J.D. and D.V. Catron. 1962. The free blood plasma amino acids of swine as related to the source of dietary proteins. J. Nutr. 76:11-16.

Rerat, A.A., Vaugelade, P., and P. Villiers. 1980. A new method for measuring the absorption of nutrients in the pig: critical examination. In: A.G. Low and I.G. Partridge (Eds.) Current Concepts of Digestion and Absorption in Pigs. National Institute for Research in Dairying, Reading, England.

Rerat, A.A. 1973. Tentative estimation of the amount of amino acids absorbed during digestion of a protein-free diet. Proc. Nutr. Soc. 32:50 Abstract.

Rerat, A.A., Corring, T. and J.P. Laplace. 1976. Protein deposition and absorption. In: D.J.A. Cole, K.N. Boorman, P.J. Buttery, D. Lewis, R.J. Neale and H. Swan (Eds.) Protein Metabolism and Nutrition. Buttersworth, London, England.

Rogers, Q.R., Chen, M.L., Peraino, C. and A.E. Harper. 1960. Observations on protein digestion *in vivo*. 3. Recovery of nitrogen from the stomach and small intestine at intervals after feeding diets containing different proteins. J. Nutr. 72:331-339.

Rolls, B.A., Porter, J.W.G. and D.R. Westgrath. 1972. The course of digestion of different food proteins in the rat. 3. The absorption of proteins given alone and with supplements of their limiting amino acids. Br. J. Nutr. 28:283-293.

Stockland, W.L., Meade, R.J., and J.W. Nordstorm. 1970. Meat and bone meal as sources of amino acids for growing swine: use of a reference diet to predict amino acid adequacy by plasma levels. J. Anim. Sci. 31:1142-1155.

Typpo, J.T., Meade, R.J., Nordstrom, J.W. and W.L. Stockland. 1970. Influence of time of fast on concentrations of free amino acids in plasma on total amino acids in liver protein of young swine. J. Anim. Sci. 31:885-893.

Waltz, O.P. 1972. The question of absorption of protein-bound or supplementary amino acids in pigs. Z. Tierphysiol., Tierernahrg. u. Fettermittelkde 29:26-28.

Windels, H.F., Meade, R.J., Nordstrom, J.W. and W.L. Stockland. 1971. Influence of source and level of dietary protein on plasma free amino acid concentrations in growing swine. J. Anim. Sci. 32:268-276.

IV. The Application of Ileal Amino Acid Digestibilities in Practical Formulation of Pig Diets.

A. Introduction

Many studies have been carried out to determine the digestibilities of amino acids in feedstuffs for pigs. In the past, the digestibilities of the amino acids were determined using the fecal analysis method originally described by Kuiken and Lyman (1948). Literature values for amino acid digestibilities determined with this method are plentiful (de Meulaere and Feldman, 1960; Olsen et al. 1968). Amino acid digestibilities determined by the fecal analysis method consider the difference between the amino acid intake and excretion in feces. However, this method does not consider the modifying action of the microflora in the large intestine on apparent protein digestion and absorption. Studies by Zebrowska (1973) showed that both intact and enzymatically hydrolyzed casein infused into the distal part of the ileum of pigs fed a protein-free diet were digested, however, the absorbed material was rapidly and completely excreted in the urine. When casein was given *per os*, the levels of free amino acids in the portal blood were high and that of urea low. Other reports clearly show that protein or amino acids infused into the large intestine make little or no contribution to the protein status of the pig (Zebrowska, 1975; Sauer, 1976; Just et al. 1981). Further evidence of the intense fermentation that occurs in the large intestine of the pig is the presence of 62-76% bacterial nitrogen, as a percentage of total nitrogen in feces, corresponding to 3.1-6.5 grams bacterial nitrogen per kilogram dry matter intake (Mason, 1980). Therefore, for the reasons outlined, the ileal analysis method rather than the fecal analysis method should be considered for determining amino acid digestibilities. Amino acid digestibilities determined by the ileal analysis method are calculated based on the intake and the amount of each amino acid passing through the distal part of the ileum. In these studies, digesta are collected from pigs fitted with a simple-T cannula or a re-entrant cannula at the distal part of the ileum.

Amino acid digestibility coefficients obtained by the fecal analysis method are, for most amino acids in most feedstuffs, higher than those obtained by the ileal analysis method (Sauer

et al., 1977; Ivan and Farrell, 1976). In some of the studies, net synthesis of methionine and lysine has been reported in the large intestine (Zebrowska et al., 1978; Low, 1982; Sauer et al., 1982). Therefore, depending on the amino acid and on the feedstuff, digestibilities obtained by the fecal analysis method over-estimate (which is usually the case) or under-estimate those obtained by the ileal analysis method. Lysine, the sulphur-containing amino acids (methionine and cystine), threonine and tryptophan can be considered the most important amino acids in practical diet formulation, as these are often first-, second- or third- limiting in many feedstuffs. Of these amino acids, cystine, threonine and tryptophan usually disappear to a large extent in the large intestine (Tanksley and Knabe, 1984).

Amino acid requirements of the pig are usually determined by growth responses to the addition of graded levels of the amino acid in question to a basal diet that is deficient in this amino acid (Cole, 1979). However, based on this criterion, the requirements will be dependant on the digestibility of the amino acid in question in the basal diet.

The objectives of the present studies were as follows:

(1.) to formulate a basal diet that would show a large difference between the supply of threonine as measured by the ileal and fecal analysis method. Among the indispensable amino acids, threonine usually disappears to the largest extent in the large intestine.

(2.) to determine the ileal and fecal digestibilities of the amino acids in the basal diet.

(3.) to determine the requirement of threonine for growing pigs, based on the supply of threonine measured by the ileal and fecal analysis method.

B. Methods and Materials

Experiment 1: Amino acid digestibilities

Two trials, involving a total of 19 cannulated and non-cannulated pigs, were conducted to determine the ileal and fecal amino acid digestibilities of two diets. In each trial, the pigs were randomly allotted to one of the dietary treatments.

The pigs were individually housed in adjustable Shinfield-type metabolic cages in an environmentally controlled barn with continuous light and an air temperature in the range of 19-21°C. Each cage was equipped with a self-feeder and water was administered with the feed at a ratio of 2 to 1.

The pigs were fed equal amounts twice daily at 0800 and 1600 hours. The amount of feed supplied was based on the individual liveweight and expected liveweight gain of each pig. The dietary intake was approximately 90% *ad libitum* (90g/kg body weight $^{0.75}$). Individual weight gain and feed intake were adjusted at weekly intervals during both trials.

The basal diet was formulated to contain 16.5% crude protein. The ingredients used to formulate the basal diet were corn, hominy feed and meat meal. Previous digestibility trials carried out at the Instituut Landbouw Onderzoek en Biochemie (I.L.O.B.) showed these ingredients to have a large difference between the supply of threonine as determined in feces and ileal digesta. A diet consisting of corn and soybean meal, with a similar protein content as the basal diet, was included as a reference. The proximate and amino acid analyses of the individual dietary ingredients used in the formulation of the basal and reference diets are shown in Table IV.1. The formulation and proximate and amino acid analyses of the complete basal and reference diets are shown in Tables IV.2 and IV.3, respectively. The amount of feed ingredients needed for completing the entire experiment was obtained and stored prior to initiating the experiment.

Trial 1: Determination of fecal amino acid digestibilities

This trial was performed with ten barrows of the Dutch Landrace x Great Yorkshire cross (initial average liveweight of 35 kg). Five pigs were allocated to one of the two dietary treatments (Table IV.3).

The pigs were allowed a minimum of 14 days to adapt to the metabolic cages and the dietary treatments. The 5 day fecal collection was initiated following the adaptation period. Feces was collected at 0800, 1200 and 1700 hours from each pig. Individual fecal samples for the total 5 day collection were weighed, pooled and subsampled. The

subsamples were stored at -23°C until further analyses.

Trial 2: Determination of ileal amino acid digestibilities

Ten barrows of the Dutch Landrace x Great Yorkshire cross (initial average liveweight of 35 kg) were surgically fitted with ileo-cecal re-entrant cannulae according to the procedures described by Easter and Tanskley (1973). The cannulae were constructed from polyvinyl chloride plastisol with a 20 mm internal diameter (Plate IV.1). Initially, there were five pigs per dietary treatment. However, due to post-surgical complications, one of the pigs fed the reference diet was removed from the trial.

A broad spectrum antibiotic was administered for 5 days following surgery and the pigs were allowed a minimum recovery period of 21 days. The adaptation period to the diet was initiated only when the pigs had fully recovered from surgery and returned to full feed and water consumption.

Three 24 hour digesta collections were made at different intervals following a 10 day adaptation period. Collection of digesta was carried out on alternate days. The re-entrant cannula was opened at the center (Plate IV.1) and polyethylene tubing, 2 metres in length and 8 cm in diameter, was connected to the proximal part of the cannula. Digesta was collected continuously through the polyethylene tubing into a collection container packed in crushed ice. During digesta collections, a rubber plug was inserted into the distal end of the cannula to prevent the backflow of digesta and to allow for the infusion of physiological saline (0.9% sodium chloride) into the cecum. In addition, the infusion of physiological saline into the cecum during the collection of digesta prevented dehydration that would result from the continuous removal of digesta. Digesta were collected hourly, weighed and frozen (-23°C) until further analyses. At the end of the collection period the samples were thawed and the digesta samples from each individual pig for the the total collection period were pooled and subsampled.

Experiment 2: Comparative growth trial

This experiment was conducted with 144 pigs of the Dutch Landrace x Great Yorkshire cross. The animals were individually fed and housed in groups of six in an artificially heated, ventilated and lighted barn. There was a ten hour light period from 0700 to 1700 hours. The pigs were housed in 2 x 3 metre straw covered concrete floored pens.

The distribution of the pigs over the treatments was performed upon their arrival to the barn and based on initial liveweight, genetic similarity and sex. The pigs were distributed among six treatments of 24 pigs each. There were two replicate pens of six females and two replicate pens of six barrows. For both sexes the average initial liveweight and their standard deviation were equalized as well as possible. At the onset of the experiment each treatment had one pen each of light females and light males (average liveweight of 15.2 kg) and heavy females and heavy males (average liveweight of 18.2 kg). A randomized block design was used to distribute the treatment groups in the barn. Upon their arrival at the barn, and prior to commencing the experiment, the pigs were dewormed with piperazine.

The experimental period lasted nine weeks, from approximately 16 kg to approximately 55 kg liveweight. The pigs were allowed 7 days to adapt to their environmental conditions at which time they were fed a starter diet with daily incremental additions of the test diets. Following the adaptation period, the pigs were fed the test diets in equal portions twice daily at 0700 and 1530 hours. The pigs were individually fed according to a feeding scheme of 90g/kg body weight $^{0.75}$. The dietary allowances were adjusted once weekly after individually weighing each pig. Water was administered with the feed at a ratio of 2 to 1.

Based on the results of Experiment 1, the reference diet was supplemented with L-lysine HCl in order to meet the ARC (1981) lysine requirements. The basal diet was supplemented with both indispensable (with the exception of threonine) and dispensable amino acids, up to the supply of amino acids, as determined with the ileal analysis method, of the reference diet (Table IV.4). Supplementation of the basal diet with other amino acids ensured that threonine would be the limiting amino acid in the basal diet. Thereafter, the basal diet,

supplemented with amino acids, was divided into five equal portions to which stepwise additions of 0.05% (by weight) threonine were added to provide dietary levels of 0.65, 0.70, 0.75 and 0.79% threonine in groups III, IV, V and VI, respectively (Table IV.5). In both diets all of the amino acids met the ARC (1981) nutrient requirements for 20 to 50 kg growing pigs. The composition of the diets is shown in Table IV.4. The proximate analyses and the amino acid additions are shown in Table IV.5.

Diet Preparation

All the feed ingredients were ground in a Wynveen hammer mill through a 1.00 mm or 1.75 mm screen for Experiments 1 and 2, respectively. After grinding, the diets were mixed in a 200 kg horizontal mixer. Two representative samples were taken each time the diets were prepared, sealed in plastic bags and stored at 4°C.

Analytical Procedures

Prior to chemical analyses, all samples were pooled, freeze-dried and ground through a 1 mm screen using a Retsch ZM 1 grinder. The dietary ingredients, feed, feces and digesta were characterized through chemical analysis for nitrogen using the Kjeldahl method according to the Association of Analytical Chemists (AOAC, 1980). Amino acid analyses were carried out by ion exchange chromatography following acid hydrolysis (6N HCl for 24 hours) using a Biotronik LC 2000 amino acid analyzer.

Dry matter determinations were performed by placing a sample in a forced-draft oven for 4 hours at 100°C. The sample was cooled in a dessication flask, reweighed and returned to the oven for a further 2 hours or until a constant dry weight was obtained.

Statistical Analysis

Responses to threonine additions were determined with a computer program Genstat using a non-linear regression calculation between threonine content in the diet and weight gain,

feed conversion efficiency and feed intake.

C. Results and Discussion

Experiment 1: Amino acid digestibilities

In these studies it was decided to use pigs fitted with re-entrant rather than with simple cannulae. The use of re-entrant cannulae allowed for a total collection of digesta. Possible problems that may occur when digestibility markers are used were avoided. However, the use of pigs fitted with re-entrant cannulae necessitates the diets be finely ground in order to prevent blockage. Therefore, the diets used in this experiment were ground through a 1 mm screen. Furthermore, the constant infusion of a physiological saline solution and the use of a re-entrant cannula with a 20 mm internal diameter, permitted unobstructed digesta flow and collection. Comparative studies on the use of re-entrant cannulae versus simple cannulae on amino acid digestibilities determined at the terminal ileum in pigs are rather limited. Zebrowska et al. (1977) and Zebrowska et al. (1977a) showed no differences (in most instances) between ileal amino acid digestibilities when either re-entrant or simple cannulae were used. However, there were significant differences ($P<0.05$) in 12 out of the 72 comparisons (4 different diets, 18 amino acids). Taverner et al. (1983) found no differences ($P>0.05$) between the dry matter, nitrogen and apparent ileal digestibilities of an *average* of amino acids in pigs fitted with re-entrant and simple cannulae that were fed a wheat, lupin and meat and bone meal diet. However, the ileal digestibilities of the *individual* amino acids were not compared in the studies by Taverner et al. (1983).

Digesta collections from the terminal ileum were made without complications. The digesta flowed easily from the proximal cannula through the collection tube into the container. The peak volume of digesta (300-400 ml per hour) usually occurred 3 to 5 hours after feeding with the intervening periods resulting in a collection of 50-100 ml per hour.

For the reference diet, with the exception of methionine, the digestibility coefficients (percent of the amino acid digested) of the amino acids were significantly ($P<0.01$) lower when determined in ileal digesta than in feces (Table IV.6). Of the indispensable amino acids, the differences (percentage units) between the ileal and fecal digestibilities ranged from 3.7 for phenylalanine to 10.9 for threonine. Of the dispensible amino acids, the differences (percentage units) ranged from 5.1 for alanine to 16.0 for glycine. Of the indispensable amino acids, the digestibility of threonine was lowest, both in ileal digesta (73.3%) and in feces (84.2%).

For the basal diet, with the exception of methionine, the digestibility coefficients of the amino acids were significantly ($P<0.01$) lower when these were determined in ileal digesta than in feces (Table IV.6). Net synthesis of methionine in the large intestine (or similar ileal and fecal digestibility coefficients) have been reported in other studies (Just, 1980; Taverner et al., 1981a; Sauer et al., 1982). Of the indispensable amino acids, the difference (percentage units) ranged from 4.9 for phenylalanine to 22.2 for threonine. Of the dispensable amino acids, the differences ranged from 8.7 for alanine to 22.6 for glutamic acid. Of the indispensible amino acids, the ileal digestibility of threonine was lowest (56.9%). Measured in feces, the digestibility of lysine was the lowest (72.8%).

The digestibilities of the amino acids were lower in the basal than in the reference diet whether measured in digesta or feces. Of the indispensable amino acids, the differences (percentage units) ranged from 16.4 for threonine to 6.8 for phenylalanine when the digestibility coefficients were determined in ileal digesta. Determined in the feces, the differences (percentage units) ranged from 17.3 for cystine to 4.0 for leucine (Table IV.6).

As a result of diet formulation, and as was expected, large differences were obtained between the digestibility of threonine as determined in ileal digesta and in feces in the reference and basal diets.

Experiment 2: Comparative growth trial

The health of the pigs throughout the trial was good although a small number of pigs was treated with antibiotics to expediate their recovery from diarrhoea. No animals died over the experimental period or were removed from the experiment.

The calculated and analyzed values of threonine in the basal diets correspond very well (Table IV.7). The addition of threonine to the basal diets had a considerable effect on weight gain and feed conversion efficiency (Table IV.8, Figures IV.1 and IV.2). Weight gain and feed conversion efficiency were significantly ($P<0.05$) lower when the threonine content of the diet was below 0.70%; no significant ($P>0.05$) improvement resulted when threonine was included above this level.

No significant ($P>0.05$) differences in weight gain and feed conversion efficiency were observed between females and barrows over the 9 week experimental period or over all 6 experimental treatments. Therefore, the results shown in Table IV.8 represent those of the combined sexes.

The differences in weight gain between the heavy and light groups were large. In general, animals with a low initial weight grew less and had a lower feed conversion efficiency than those with a high initial weight. Despite the formulation of the isonitrogenous and isocaloric reference and basal diets and the additional supply of amino acids to the basal diet up to the ileal supply of the reference diet, weight gain and feed conversion efficiency of the pigs fed the reference diet was more favourable than those fed the basal diet. This was due to the lower feed intake (Table IV.8 and Figure IV.3) and palatibility of the basal diets. Pigs fed the reference diet grew faster and more efficiently ($P<0.05$) than those fed the basal diets. Significant ($P<0.05$) differences in weight gain and feed conversion efficiency were found between treatment groups I, II and III. There were no significant ($P>0.05$) differences between treatments IV, V, and VI (Table IV.9).

No significant ($P>0.05$) differences were observed with respect to sex, weight class or sex x weight class for feed conversion efficiency. Pigs on treatment I had a significantly lower

($P<0.05$) feed conversion efficiency than those on any other treatment group except for those on treatment VI. Treatment group II had the highest feed conversion efficiency followed by treatment groups III, IV and V (Table IV.9).

The inclusion of synthetic amino acids adds to the cost of diet preparations and thus it would be desirable to formulate diets in which supplemental amino acids would be utilized to their fullest possible extent. Lysine, threonine, tryptophan and the sulphur containing amino acids are often the most limiting amino acids in cereal based diets for pigs and may be supplemented. Amino acid supplementation is based on requirements established by the NRC (1979), ARC (1981) or AEC (1978). However, these requirements are determined by growth responses to stepwise additions of the limiting amino acid to a basal diet and in view of the results of the present experiment, an accurate estimation of the requirement of the amino acid in question requires an estimate of the ileal digestibility of this amino acid in a basal diet.

In the present studies, the basal diet was formulated to show a large difference between the supply of threonine, as measured by the ileal and fecal analysis method. The ileal and fecal digestibilities of threonine were 56.9 and 79.1%, respectively. On a quantitative basis, this difference amounts to 1.4 g/kg dry matter. The total supply and the supply based on the ileal and fecal analysis method are shown in Table IV.7. In these calculations, the digestibility of synthetic threonine was assumed to be 100.0%. This was based on results by Buraczewska et al. (1978) who found the digestibility of synthetic lysine to be 100.0%.

For growing pigs in the 20 kg liveweight range, the ingredients used in the proportions to formulate diets II, III, IV, V and VI initially presented palatability problems and consequently there were feed refusals and diarrhoea for the first three weeks of the trial. This is shown in the poor weight gain, feed conversion efficiency and feed intake (Figures IV.1, IV.2 and IV.3, respectively). This was less evident as the pigs grew larger because the diet appeared to be more palatable and digestible. The cumulative weight gain of the pigs on each of the dietary treatments is shown in Figure IV.4.

Average daily gain was not improved ($P>0.05$) when the threonine level in the diet exceeded 0.70% in the present studies. Based on weight gain and feed conversion efficiency, Berende and Bertram (1983) suggest the threonine requirement of pigs weighing 15 to 45 kg to range from 0.55 to 0.63% of the diet. However, previous results by Taylor et al. (1981) have shown an improvement in weight gain and feed conversion efficiency of female pigs weighing 25 to 55 kg when the threonine content of the diet was increased from 0.47 to 0.67%. On the other hand, Sowers and Meade (1972) found 0.39 and 0.32% threonine in a corn based diet to result in optimum average daily gain and feed conversion efficiency, respectively. Contrary to the threonine requirements expressed by Berende and Bertram (1983), Taylor et al. (1981) and Sowers and Meade (1972), a survey of the literature by Homb (1976) suggests a threonine requirement for grower pigs weighing 20 to 50 kg from 0.45 to 0.50%. Recent American data (Cohen and Tanskley, 1977) suggest an optimum threonine content for grower pigs with an average initial weight of 17.7 kg to be 0.47% of the diet. Schuller and Voigt (1976) reported that dietary threonine levels of 0.41 to 0.47% in a 15 to 17% crude protein diet were sufficient to meet the requirements of growing pigs weighing 12 to 35 kg. Jensen (1981) and Aw-Yong and Beames (1975), on the other hand, recommend threonine levels in the diet for growing pigs to be 0.45% and 0.57%, respectively.

Discrepancies in the threonine requirement of growing pigs are evident between European and American data. The ARC (1981) literature survey suggests the threonine requirement for growing pigs to range from 0.56 to 0.60% of the diet. This is considerably higher than the threonine content of 0.45% recommended by the NRC (1979) for 20 to 35 kg pigs fed a fortified grain-SBM diet *ad libitum*. The French AEC (1978) recommends a dietary threonine content of 0.41% for growing pigs weighing 20 to 55 kg which is lower than both the ARC (1981) and NRC (1979) recommendations. Possible explanations for these discrepancies should be addressed. The major reason for these differences may well relate to differences in the digestibility of threonine in the basal diet. For example, in the present studies, the ileal digestibility of threonine was low in the basal diet, 56.9% (Table IV.6). Consequently, the

total requirement of threonine was relatively high, 0.70-0.79% (Tables IV.7 and IV.9). However, based on the supply of threonine as measured by the ileal analysis method, the requirement was considerably less and ranged from 0.39 to 0.49%. Based on the fecal analysis method, the requirement was intermediate and ranged from 0.52 to 0.62%. In addition, the present studies show that requirements for threonine should be related to the supply of threonine determined in ileal digesta and not in feces, as shown by the large differences in the requirement of threonine as determined by both methods.

In conclusion, amino acid digestibilities determined by the ileal analysis method appear to reflect the amino acids available to the pig and should be considered for accurate diet formulation. Furthermore, in the assessment of amino acid requirements, the digestibility of the amino acid in question in the basal diet should be taken into account.

Table IV.1 Proximate and amino acid analyses of the dietary ingredients.

Ingredient	Hominy feed	Corn	Meat meal	Soybean meal
Proximate analyses, %				
Crude protein, N x 6.25	13.38	8.64	57.99	45.70
Dry matter	91.05	87.62	91.66	88.08
Amino acids[1, 2], %				
Indispensable				
Arginine	0.84	0.36	3.59	3.20
Histidine	0.36	0.23	1.18	1.17
Isoleucine	0.45	0.33	1.79	2.26
Leucine	1.10	1.14	4.49	3.60
Lysine	0.61	0.31	3.07	2.96
Phenylalanine	0.55	0.43	2.36	2.31
Threonine	0.54	0.34	2.41	1.95
Tyrosine	0.45	0.38	1.54	1.75
Valine	0.71	0.46	3.56	2.33
Dispensable				
Alanine	0.84	0.67	3.78	1.98
Aspartic acid	1.01	0.58	4.48	5.36
Glutamic acid	1.96	1.70	6.65	8.59
Glycine	0.68	0.31	5.78	1.92
Proline	0.94	0.92	5.25	2.96
Serine	0.69	0.49	3.99	2.69

[1]Expressed on a dry matter basis.
[2]Cystine, methionine and tryptophan were not determined.

Table IV.2 Formulation of the diets (Experiment 1).

Ingredients, %	Reference (%)	Basal (%)
Corn	76.06	48.50
Hominy feed	—	40.00
Soybean meal	20.91	—
Meat meal	—	10.70
Calcium monohydrophosphate	1.50	—
Calcium carbonate	1.00	—
Mineral[1]/vitamin[2] mix	0.50	0.50
Iodized salt	0.30	0.30
Total	100.00	100.00

[1]The mineral mixture contributed the following per kilogram: 12,000 mg copper; 16,000 mg iron; 10,000 mg zinc; 4,800 mg manganese; 80 mg cobalt; 80 mg iodine; 20 mg selenium.
[2]The vitamin mixture contributed the following per kilogram: 1,000,000 IU vitamin A; 200,000 IU vitamin D_3; 700 mg vitamin B_2; 3,600 mg niacin; 1,000 mg pantothenic acid; 3 mg vitamin B_{12}; 2,000 IU vitamin E; 80 mg vitamin K_3.

Table IV.3 Proximate and amino acid analyses of the diets (Experiment 1).

	Reference	Basal
Proximate analyses, %		
Crude protein, N x 6.25	16.27	16.38
Dry matter	88.72	88.75
Net energy (kj/kg), calculated	9828	9807
Amino acids[1], %		
Indispensable, analyzed[2]		
Arginine	0.99	0.98
Histidine	0.43	0.37
Isoleucine	0.73	0.57
Leucine	1.58	1.44
Lysine	0.78	0.63
Methionine	0.33	0.30
Cystine	0.34	0.39
Phenylalanine	0.81	0.64
Threonine	0.67	0.63
Tyrosine	0.62	0.52
Valine	0.83	0.85
Dispensable		
Alanine	0.88	0.99
Aspartic acid	1.51	1.08
Glutamic acid	3.11	2.37
Glycine	0.65	1.00
Proline	1.13	1.37
Serine	0.92	0.89

[1]Expressed on a dry matter basis.
[2]Tryptophan was not determined.

Table IV.4 Formulation of the diets (Experiment 2).

Ingredient, %	Reference	Basal
Corn	71.74	38.23
Hominy feed	—	40.00
Meat meal	—	10.70
Soybean meal	20.91	—
Amino acid mix[1]	4.00	12.00
Calcium monohydrophosphate	1.87	—
Calcium carbonate	0.68	0.27
Mineral[2]/Vitamin[3] mix	0.50	0.50
Iodized salt	0.30	0.30
Total	100.00	100.00

[1]The amino acid mixture supplied per kilogram of the basal diet: 1.0 g L-arginine; 0.9 g L-histidine; 1.9 g L-isoleucine; 2.1 g L-leucine; 5.3 g L-lysine; 0.5 g L-methionine; 0.6 g L-cystine; 1.8 g L-phenylalanine; 0, 0.5, 1.0, 1.5 or 2.0 g L-threonine (basal diets 1 to 5); 1.3 g L-tyrosine; 0.8 g L-tryptophan; 0.8 g L-valine; 5.2 g L-aspartic acid; 9.9 L-glutamic acid; 1.8 g L-glycine and 1.0 g L-serine. The non-essential amino acids were added to make all diets isonitrogenous. Corn was used as the premix base. The amino acid mixture supplied per kilogram of the reference diet: 3.2 g L-lysine. Hominy feed was used as the premix base.
[2]The mineral mixture contributed the following per kilogram: 12,000 mg copper; 16,000 mg iron; 10,000 mg zinc; 4,800 mg manganese; 80 mg cobalt; 80 mg iodine; 20 mg selenium.
[3]The vitamin mixture contributed the following per kilogram: 1,000,000 IU vitamin A; 200,000 IU vitamin D_3; 700 mg vitamin B_2; 3,600 mg niacin; 1,000 mg panthothenic acid; 3 mg vitamin B_{12}; 2,000 IU vitamin E; 80 mg vitamin K_3.

Table IV.5 Proximate and amino acid analyses of the diets (Experiment 2).

	Reference	Basal
Proximate analyses, %		
Crude protein, N x 6.25	16.27	16.38
Dry matter	88.72	88.75
Net energy (kj/kg), calculated	9828	9807
Amino acids[1], %		
Indispensable[2]		
Arginine	0.99	0.98
Histidine	0.43	0.50
Isoleucine	0.71	0.69
Leucine	1.60	1.58
Lysine	1.12	1.19
Methionine	0.29	0.33
Cystine	0.35	0.33
Phenylalanine	0.81	0.82
Threonine[3]	0.67	0.60
Tyrosine	0.61	0.60
Valine	0.81	0.88
Dispensable		
Alanine	0.92	1.06
Aspartic acid	1.50	1.60
Glutamic acid	3.17	3.32
Glycine	0.64	1.22
Proline	0.98	0.95
Serine	0.89	0.83

[1]Expressed on a dry matter basis.
[2]Tryptophan was not determined.
[3]Threonine additions are shown in Table IV.4.

Table IV.6 Ileal and fecal digestibility coefficients (Experiment 1).

Location	Reference		Basal	
	Ileal Digesta[1]	Feces[2]	Ileal Digesta[1]	Feces[2]
Crude protein	76.8 ± 2.6*a*[3]	86.3 ± 2.1*b*	64.7 ± 4.1*A*[4]	77.5 ± 1.5*B*
Dry matter	75.7 ± 2.0*a*	86.8 ± 1.0*b*	70.8 ± 2.5*A*	81.8 ± 1.6*B*
Amino acids				
Indispensable[5]				
Arginine	88.8 ± 1.7*a*	92.4 ± 1.4*b*	79.9 ± 2.6*A*	85.1 ± 1.2*B*
Histidine	81.3 ± 3.3*a*	91.9 ± 1.5*b*	71.1 ± 3.5*A*	85.7 ± 1.4*B*
Isoleucine	82.4 ± 2.9*a*	86.0 ± 2.8*b*	71.6 ± 4.9*A*	76.7 ± 1.8*B*
Leucine	85.9 ± 2.5*a*	89.8 ± 2.0*b*	75.9 ± 4.0*A*	85.5 ± 1.1*B*
Lysine	80.1 ± 3.4*a*	85.2 ± 2.7*b*	64.6 ± 3.7*A*	72.8 ± 2.4*B*
Methionine	88.0 ± 2.2*a*	87.3 ± 2.3*a*	80.7 ± 4.0*A*	80.0 ± 1.6*A*
Cystine	76.1 ± 3.7*a*	89.0 ± 2.1*b*	51.1 ± 6.8*A*	71.7 ± 1.9*B*
Phenylalanine	84.3 ± 2.6*a*	88.0 ± 2.2*b*	77.5 ± 3.9*A*	82.4 ± 1.3*B*
Threonine	73.3 ± 3.8*a*	84.2 ± 2.7*b*	56.9 ± 5.4*A*	79.1 ± 2.2*B*
Tyrosine	83.6 ± 2.9*a*	87.4 ± 2.4*b*	74.9 ± 3.6*A*	80.0 ± 1.3*B*
Valine	79.1 ± 3.6*a*	85.7 ± 2.7*b*	68.3 ± 4.8*A*	77.4 ± 1.7*B*
Dispensable				
Alanine	81.0 ± 3.5*a*	86.1 ± 2.7*b*	73.4 ± 4.3*A*	82.1 ± 1.3*B*
Aspartic acid	80.1 ± 3.0*a*	87.8 ± 2.3*b*	54.3 ± 5.2*A*	75.6 ± 2.0*B*
Glutamic acid	86.4 ± 2.7*a*	92.2 ± 1.5*b*	71.6 ± 3.8*A*	82.4 ± 1.4*B*
Glycine	68.2 ± 4.0*a*	84.2 ± 2.2*b*	58.7 ± 3.5*A*	81.3 ± 1.4*B*
Proline	78.2 ± 3.7*a*	92.1 ± 1.0*b*	67.1 ± 3.0*A*	85.0 ± 1.8*B*
Serine	80.7 ± 2.7*a*	89.1 ± 1.6*b*	62.5 ± 3.9*A*	78.0 ± 1.8*B*

[1]Means ± SD for 9 observations.
[2]Means ± SD for 10 observations.
[3]Means within the same row with different subscripts differ ($P<0.01$).
[4]Means within the same row with different subscripts differ ($P<0.01$).
[5]Tryptophan was not determined.

Table IV.7 Dietary treatments (Experiment 2).

Group	Diet	Crude Protein (N x 6.25%)	Addition of Threonine (%)	Threonine supply in the diets (%)			
				Total		Ileal Digesta[1]	Feces[2]
				Calculated	Analyzed		
I	Reference	16.3	0.00	0.67	0.67	0.49	0.56
II	Basal	16.4	0.00	0.63	0.60	0.34	0.47
III	Basal	16.4	0.05	0.68	0.65	0.39	0.52
IV	Basal	16.4	0.10	0.73	0.70	0.44	0.57
V	Basal	16.4	0.15	0.78	0.75	0.49	0.62
VI	Basal	16.4	0.20	0.83	0.79	0.53	0.66

[1]Collected via the ileal analysis method.
[2]Collected via the fecal analysis method.

Table IV.8 Effect of added threonine on weight gain, feed intake and feed conversion efficiency after 9 weeks (Experiment 2).

Treatment	Reference			Basal		
Group	I	II	III	IV	V	VI
Threonine in diets (%)	0.67	0.60	0.65	0.70	0.75	0.79
				Combined Sexes[1]		
Initial weight (kg)	16.8 ± 2.0	16.8 ± 2.0	16.8 ± 2.1	16.8 ± 1.9	16.8 ± 1.9	16.8 ±1.9
Weight gain						
total (kg)	39.4 ± 1.6	20.9 ± 5.0	25.5 ± 3.3	29.6 ± 2.4	30.0 ± 2.1	32.2 ± 4.4
per day (g)	625 ± 25.7	332 ± 79.6	405 ± 53.2	470 ± 37.9	476 ± 32.7	511 ± 69.9
Feed intake per day (g)	1360	1072	1140	1202	1198	1211
Feed conversion efficiency (kg feed/kg gain)	2.19 ± 0.08	3.33 ± 0.37	2.92 ± 0.19	2.58 ± 0.15	2.61 ± 0.13	2.43 ± 0.12

[1]Means ± SD.

Table IV.9 Average daily gain and feed conversion efficiency after 9 weeks (Experiment 2).

Group	ADG, grams	FCE
I	625 ± 5.25*d*	2.19 ± 0.01*d*
II	332 ± 16.24*a*	3.33 ± 0.07*a*
III	405 ± 10.86*b*	2.92 ± 0.04*b*
IV	470 ± 7.74*c*	2.58 ± 0.03*bc*
V	476 ± 6.67*c*	2.61 ± 0.02*bc*
VI	511 ± 14.27*c*	2.43 ± 0.02*cd*

a,b,c,d Means ± SE within column and with different letters differ significantly ($P<0.05$).

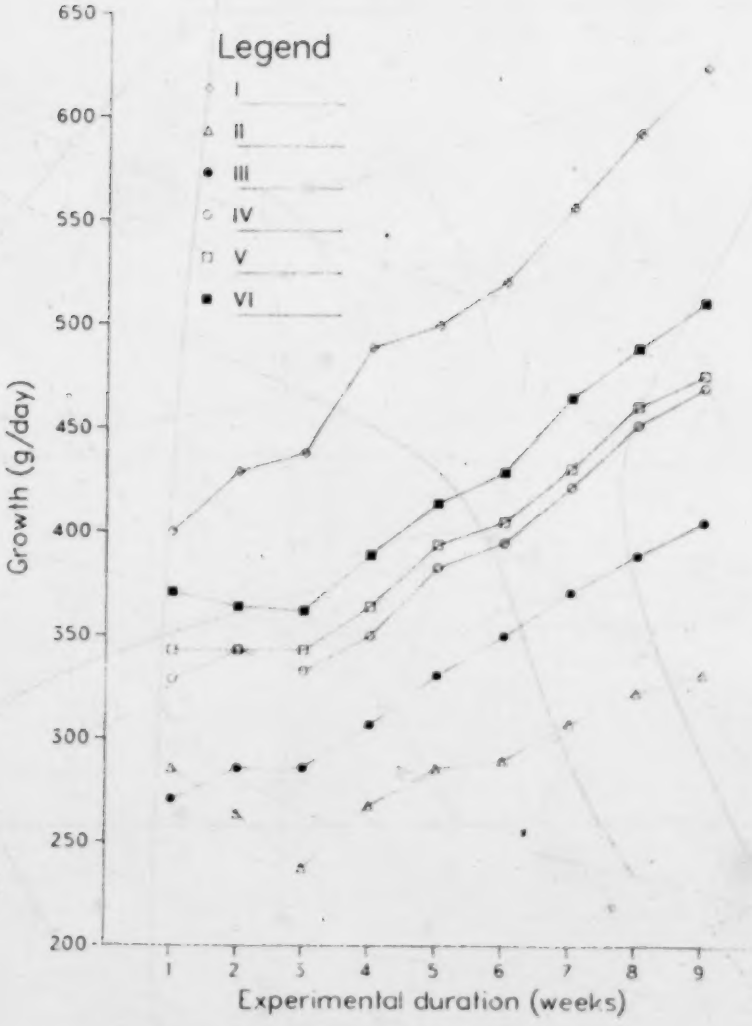


Figure IV.1 Average daily gain responses of the treatment groups to the addition of threonine.

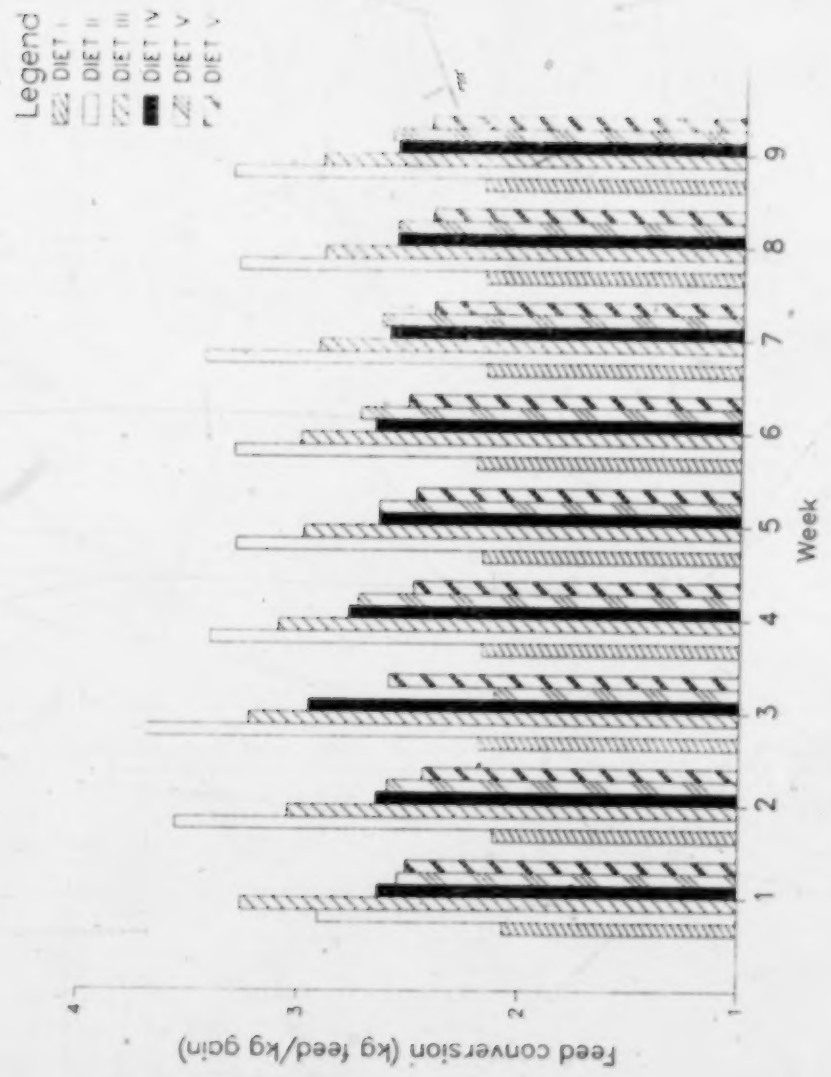


Figure IV.2 Feed conversion efficiencies of each of the treatment groups.

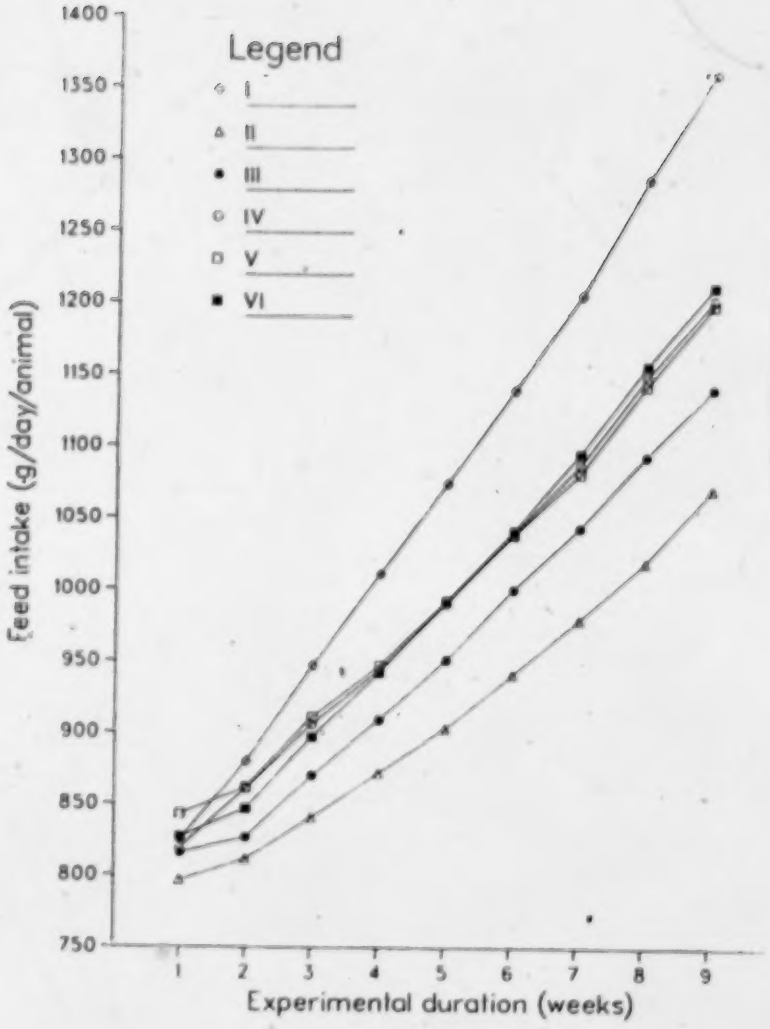


Figure IV.3 Feed intake of each of the treatment groups.

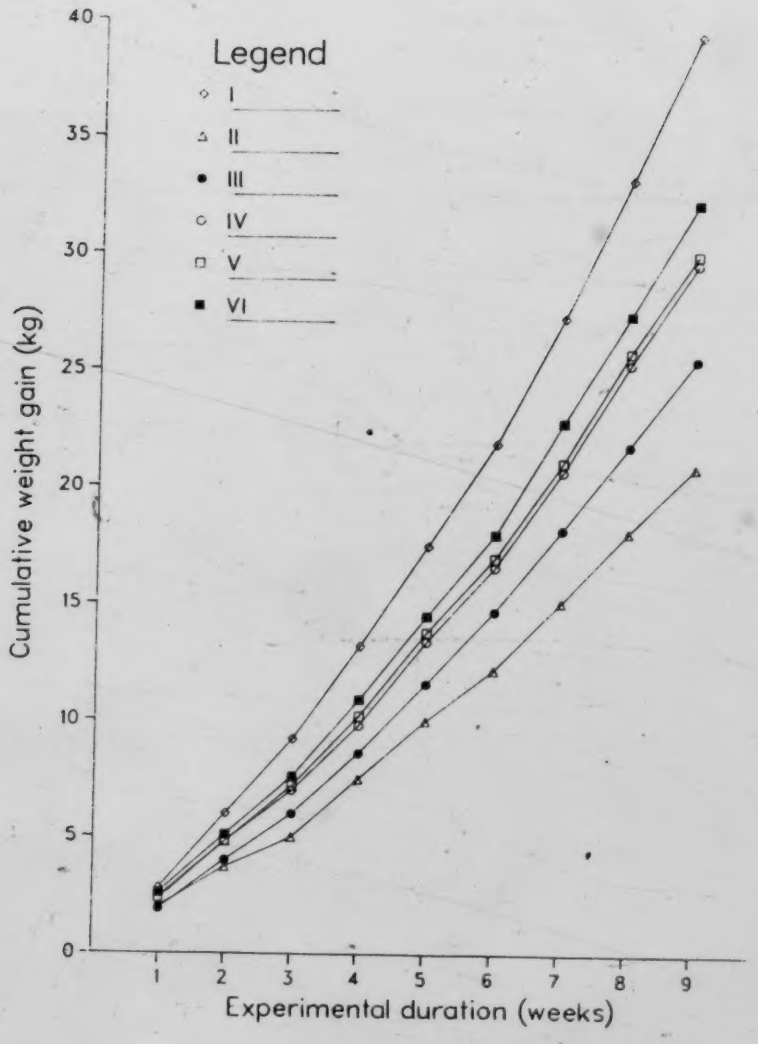
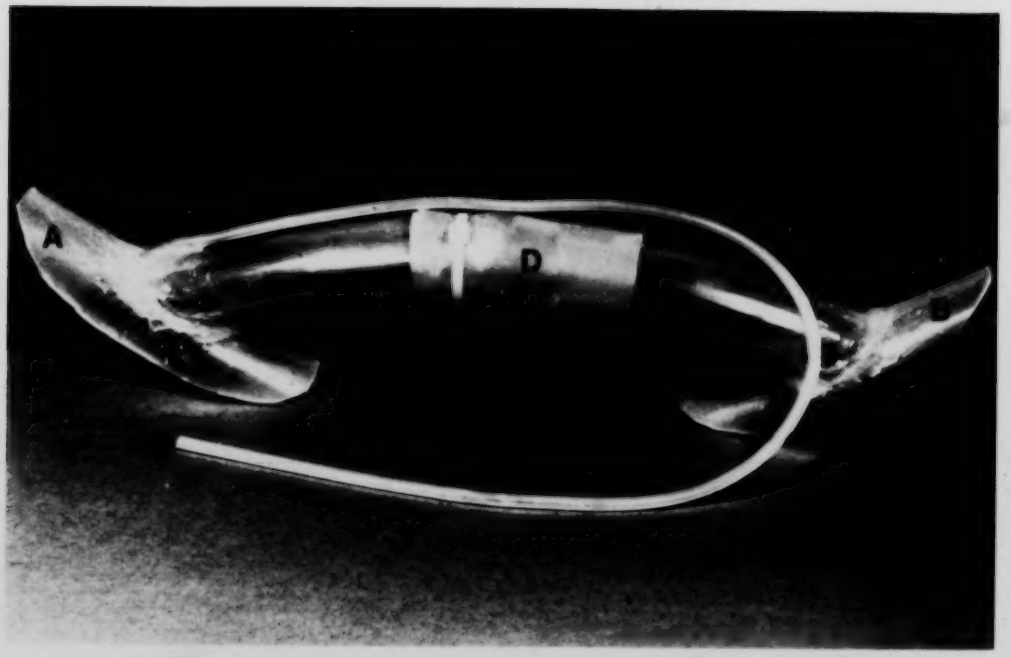


Figure IV.4 Cumulative weight gain of each of the treatment groups.



A. The part of the re-entrant cannula inserted into the distal end of the ileum.

B. The part of the re-entrant cannula inserted into the cecum.

C. Tubing through which physiological saline was infused.

D. Tubing connecting the proximal and distal parts of the cannula.

Plate IV.1 Ileo-cecal re-entrant cannula.

D. Literature Cited

A.E.C. 1978. Animal Feeding: Energy, Amino Acids, Vitamins, Minerals. Document Number 4 A.E.C.; Commentry, France.

A.O.A.C. 1980. Official Methods of Analysis. (13th Ed.). Association of Offical Analytical Chemists. Washington, D.C., U.S.A.

Agriculture Research Council. 1981. The nutrient requirement of farm livestock. Number 3. Pigs. Technical reviews and summaries. Agricultural Research Council. London, England.

Aw-Yong, L.M. and R.M. Beames. 1975. Threonine as a second limiting amino acid in Peace River barley for growing-finishing pigs and growing rats. Can. J. Anim. Sci. 55:765-783.

Berende, P.L.M. and H.L. Betram. 1983. Threonine requirement of young pigs. Z. Tierphysiol., Tierernahrg. u. Futtermittelkde 49:30-38.

Buracewska, L., Zebrowska, T. and S. Buraczewska. 1978. The rate of passage of synthetic lysine and dietary protein in the upper half of the small intestine in pigs. Rocz. Nauk. Roln. Ser. B Zootech. 99:107-113.

Cohen, R.S. and T.D. Tanksley, Jr. 1977. Threonine requirement of growing and finishing swine fed sorghum-soybean meal diets. J. Anim. Sci. 45:1079-1083.

Cole, D.J.A. 1979. Amino acid nutrition of the pig. In: W. Haresign and D. Lewis (Eds.) Recent Advances in Animal Nutrition. Butterworths, London, England.

de Meulaere, H.J. and R. Feldman. 1960. Availability of amino acids in maize. J. Nutr. 72:447-450. 15:310-317.

Easter, R.A. and T.D. Tanksley. 1973. A technique for re-entrant ileocecal cannulation for swine. J. Anim. Sci. 36:1099-1103.

Fuller, M.F., Mennie, I. and R.M. Crofts. 1979. The amino acid supplementation of barley for the growing pig. 2. Optimal additions of lysine and threonine for growth. Br. J. Nutr. 41:333-340.

Holmes, J.G.H., Baley, H.S., Leadbeater, P.A. and F.D. Horney. 1974. Digestion of protein in the small and large intestine of the pig. Br. J. Nutr. 32:479-489.

Homb, T. 1976. In: D.J.A. Cole (Ed.) Protein Metabolism and Nutrition. Buttersworth, London, England.

Ivan, M. and D.J. Farrell. 1976. Nutritional evaluation of wheat. 5. Disappearance of components in digesta of pigs prepared with two re-entrant cannulae. Anim. Prod. 23:111-119.

Jensen, A.H. 1981. Dietary nutrient allowances for swine. Feedstuffs Reference Issue 52(30):33-34, 36 and 38.

Just, A. 1980. Ileal digestibility of protein: applied aspects. In: A.G. Low and I.G. Partridge (Eds.) Current Concepts of Digestion and Absorption in Pigs. National Institue for Research in Dairying, Reading, England.

Just, A., Jorgensen, H. and J.A. Fernandez. 1981. The digestive capacity of the caecum-colon and the value of nitrogen absorbed from the hindgut for protein synthesis in pigs. Br. J. Nutr. 32:479-480.

Kuiken, K.A. and C.M. Lyman. 1948. Availability of amino acids in some foods. J. Nutr. 36:359-368.

Low, A.G. 1982. Digestibility and availability of amino acids from feedstuffs for pigs: a review. Livestock Prod. Sci. 9:511-520.

Mason, V.C. 1980. Role of the large intestine in the processes of digestion and absorption in the pig. In: A.G. Low and I.G. Partridge (Eds.) Current Concepts of Digestion and Absorption in Pigs. National Institute for Research in Dairying, Reading, England.

National Reseach Council. 1979. Nutrient Requirement of Swine. Eighth revised edition. Washington, D.C., U.S.A.

Olsen, E.M., Summers, J.D. and S.J. Slinger. 1968. Evaluation of protein quality in wheat by-products: digestibility of protein and absorption of amino acids by the rat. Can. J. Anim. Sci. 48:215-220.

Sauer, W.C. 1976. Factors influencing amino acid availability for cereal grains and their components for growing monogastric animals. PhD. Thesis. University of Manitoba, Winnipeg, Manitoba.

Sauer, W.C., Strothers, S.C. and R.J. Parker. 1977. Apparent and true availabilities of amino acids in wheat and milling by-products for growing pigs. Can. J. Anim. Sci. 57:775-784.

Sauer, W.C., Cichon, R. and R. Misir. 1982. Amino acid availability and protein quality of canola and rapeseed meal for pigs and rats. J. Anim. Sci. 54:292-301.

Schuler, D. and C. Voigt. 1976. Untersuchungen zum einsatz von synthetischem threonin bei wachsenden schweinen. Arch. Tierernahrung Bd. 26 H 8:569-574.

Sowers, J.E. and R.J. Meade. 1972. Threonine requirement of the growing pig. J. Anim. Sci. 35:244 Abstract.

Tanskley, T.D. and D.A. Knabe. 1984. Ileal digestibilities of amino acids in pig feeds and their use in formulating diets. In: W. Haresign and D.J.A. Cole (Eds.) Recent Advances in Animal Nutrition. Buttersworth, London, England.

Taverner, M.R., Hume, I.D. and D.J. Farrell. 1981a. Availability to pigs of amino acids in cereal grains. 1. Endogenous levels of amino acids in ileal digesta and feces of pigs given cereal diets. Br. J. Nutr. 46:149-158.

Taverner, M.R., Curic, D.M. and C.J. Rayner. 1983. A comparison of the extent and site of energy and protein digestion of wheat, lupin and meat and bone meal by pigs. J. Sci. Food. Agri. 34:122-128.

Taylor, A.J., Cole, D.J.A. and D. Lewis. 1981. Amino acid requirements of growing pigs. 3. Threonine. Anim. Prod. 34:1-8.

Zebrowska, T. 1973. Digestion and absorption of nitrogenous compounds in the large intestine of the pig. Rocz. Nauk. Roln. Ser. B Zootech. 95:85-90.

Zebrowska, T. 1975. The apparent digestibility of nitrogen and individual amino acids in the large intestine of the pig. Rocz. Nauk. Roln. Ser. B Zootech. 97:117-123.

Zebrowska, T., Buraczewska, L. and S. Buraczewski. 1977. Comparision of apparent digestibility of amino acids in the small intestine and the whole digestive tract of pigs. Vth Int. Symp. on Amino Acids. Budapest, Hungary.

Zebrowska, T., Buraczewska, L., Pastuszewska, B., Chamberlain, A.G. and S. Buraczewski. 1977a. Effect of diet and method of collection on amino acid composition of digesta and on digestibility of nitrogen and amino acids in pigs. Vth Int. Symp. on Amino Acids. Budapest, Hungary.

Zebrowska, T., Buraczewska, L. and S. Buraczewski. 1978. The apparent digestibility of amino acids in the small intestine and the whole digestive tract of pigs fed diets containing different sources of protein. Rocz. Nauk. Roln. Ser. B Zootech. 99:87-98.

V. Conclusion

Studies were carried out to surgically prepare pigs with permanent indwelling portal vein catheters that would remain patent for 21 days and allow for continuous blood sampling from conscious and unrestrained pigs. The success of venous catheterization was a function of the surgical procedure and the material used for catheter construction. The proposed catheter design should rectify some of the problems previously associated with catheter malfunction. However, there is a need to find new material for catheter construction that will allow for prolonged blood collection. Furthermore, studies were carried out to determine in pigs fitted with a catheter in the portal vein, whether lysine concentrations and time of maximum lysine appearance in the portal blood differed when either all the dietary lysine was supplied in the protein bound form or when a portion of the dietary lysine was supplied as synthetic lysine. Although there was considerable variation in the results among pigs and between pigs on the same dietary treatment, there seemed to be no difference between portal blood lysine concentrations and time of maximum lysine appearance when pigs were fed lysine in the form of protein or when a portion of the dietary lysine was derived from supplemental lysine.

In the last experiment, studies were carried out to determine the threonine requirements of growing pigs. A basal diet was formulated that showed a large difference between the digestible threonine content as determined by the ileal and fecal analysis method. These studies showed that amino acid digestibilities determined by the ileal analysis method should be considered for accurate diet formulation. In addition, in the assessment of threonine requirements, the digestibility of threonine as determined by the ileal analysis method should be taken into account.